İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

THE IMPACT OF ULTRASOUND PRETREATMENT ON PERFORMANCE OF ANAEROBIC DIGESTION OF RESIDUAL BIOMASS OF A FARMLAND BIOGAS PLANT

M.Sc. Thesis by
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Department : Environmental Engineering

Programme : Environmental Biotechnology

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★FEN BİLİMLERİ ENSTİTÜSÜ

ULTRASONİK ÖN ARITMANIN TARIMSAL BİYOGAZ TESİSLERİNDEN ÇIKAN ARTIK BİYOKÜTLENİN ANAEROBİK ÇÜRÜTME VERİMİNE ETKİSİ

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FOREWORD

First of all I would like to thank Prof. Dr. Ing. Uwe Neis for his guidance, valuable advises, ultimate support and kindness during this study. Second, I would like to thank my supervisor Prof. Dr. Rüya Taşlı Toraman, for her support and patience in each step of this study. I am also greatful to Dr.-Ing Klaus Nickel and Dr. Silvana Velten for their guidance and never ending patience in answering my questions, Susanne Eggers for her assistance and support at laboratory and Dipl. Ing. Julian Tiencken for his friendly support at Ultrawaves GmbH.

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Environmental Biotechnology

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ABBREVIATIONS

AD	: Anaerobic Digestion
BMP	: Biomethane Potential
COD	: Chemical Oxygen Demand
DD	: Disintegration Degree
NH4-N	: Ammonia Nitrogen
NL	: Normalized Litres
Nml	: Normalized Millilitres
OUR	: Oxygen Uptake Rate
SCOD	: Soluble Chemical Oxygen Demand
SE	: Specific Energy
TS	: Total Solids
TOC	: Total Organic Carbon
TN	: Total Nitrogen
US	: Ultrasonication
WWTP	: Wastewater Treatment Plant
VDI	: Verein Deutscher Ingenieure
VFA	: Volatile Fatty Acids
VS	: Volatile Solids

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THE IMPACT OF ULTRASOUND PRE-TREATMENT ON PERFORMANCE OF ANAEROBIC DIGESTION OF RESIDUAL BIOMAS OF A FARMLAND BIOGAS PLANT

SUMMARY

Residual biomass after conventional anaerobic digestion on sewage treatment or farm land biogas plants contains varying amounts of cellulose, hemi–cellulose and lignin. In recent years, production of the second-generation bio-products such as bio-ethanol, biodiesel, methane and bio-hydrogen from lignocellulosic biomass is increasingly being preferred than from energy crops. The main reason for this tendency is, energy crops compete for land and water with food crops that are already in high demand. However, many factors, like lignin content, crystallinity of cellulose, and particle size, limit the digestibility of hemicellulose and cellulose present in the lignocellulosic biomass.

In this study, the impact of low frequency high-power ultrasound treatment on the performance of anaerobic digestion on the residual biomass from the secondary digester of a farm land biogas plant is investigated.

The sludge taken from secondary digestion tank of a farmland biogas plant was sonicated for varying durations. Untreated and sonicated samples were kept in anaerobic conditions for 40 days and biogas productions of the samples were monitored. Same prosedure was also applied to the samples from anaerobic digester of a wastewater treatment plant and the results were compared.

The experimental results showed that, anaerobic digestion efficiency of the residual biomass of farmland biogas plant increased with increasing specific energy of ultrasonic pre treatment. 2334 kJ/kgTS specific energy caused 21 % increase in cumulative biogas production. The results of the tests with the residual biomass wastewater treatment showed that the effect of ultrasonic treatment on anaerobic digestion efficiency of this biomass is higher and anaerobic digestion rate of this biomass was larger. Specific energies 1122 kJ/kgTS and 5413 kJ/kgTS was applied to this biomass and it was observed that 90 % of biogas production completed within 13 and 10 days respectively, and at 5413 kJ/kgTS specific energy, gas production increased by 30 percent. On the other hand, the biogas production of residual biomass of the farmland biogas plant did not reach the maximum level at the end of 40 days at any of the specific eneriges applied, but the biogas production rate started to decrease. This result supported that the anaerobic digestibility of residual biogas of farmland biogas plant was less than that of wastewater treatment plant due to the high lignin content.

ULTRASONİK ÖN ARITMANIN TARIMSAL BİYOGAZ TESİSLERİNDEN ÇIKAN ARTIK BİYOKÜTLENİN ANAEROBİK ÇÜRÜTME VERİMİNE ETKİSİ

ÖZET

Atıksu arıtma tesislerinden sonra veya tarımsal biyogaz tesislerinde uygulanan anaerobik çürütme prosesinde oluşan artık biyokütle çeşitli miktarlarda selüloz, hemi-selüloz ve lignin içermektedir. Son yıllarda, biyo-etanol, biyodizel, metan ve biyo-hidrojen gibi ikinci nesil biyo-ürünlerin üretiminde enerji ekinlerinden ziyade lignoselülozik biyokütlenin kullanılmasının tercihi artış göstermektedir. Bu eğilimin temel nedeni olarak enerji ekinlerinin hale hazırda yüksek talep gören yiyecek ekinleri ile toprak ve su rekabeti gösterilebilir. Ancak, lignin içeriği, selülozun kristalleşmesi, tanecik boyutu gibi bir çok faktör lignoselülozik biyokütlenin içinde bulunan hemiselüloz ve selülozun çürütülme verimini sınırlamatadır.

Bu çalışmada, yükek enerjili, düşük frekanslı ultrasonik ön arıtma yönteminin tarımsal biyogaz tesisinin ikincil çürütme tankından çıkan artık biyokütlenin tekrar çürütülme verimine olabilecek katkısının belirlenmesi amaçlanmıştır.

Tarımsal biyogaz üretim tesisinin ikincil çürütme tankından alınan çamura çeşitli sürelerle ultrases uygulanmıştır. Ön arıtma uygulanan ve uygulanmayan çamur örnekleri 40 gün süreyle anaerobik şartlarda bekletilerek biyogaz üretimleri izlenmiştir. Aynı işlem atıksu arıtma tesisi anaerobik çürütücüsünden çıkan artık biyokütleye de uygulanarak sonuçlar karşılaştırılmıştır.

Elde edilen deneysel sonuçlar tarımsal biyogaz üretim tesisi artık biyokütlesinin anaerobik çürütme veriminin artan ultrasound spesifik enerjisi ile artış gösrerdiğini ortaya koymuştur. Toplam biyogaz üretiminde 2334 kJ/kgTS spesifik enerjide yüzde 21 artış gözlenmiştir. Atıksu arıtma tesisi anaerobik çürütme prosesinden çıkan artık biyokütle ile yapılan deney sonucunda, ultrasonik ön arıtmanın bu biyokütlenin çürütme veriminin üzerinde daha büyük etkisinin olduğu ve bu biyokütlenin anaerobik çürütme hızının daha fazla olduğu gözlenmiştir. Bu çamura 1122 kJ/kgTS ve 5413 kJ/kgTS değerinde spesifik enerji uygulanmış biyogaz üretiminin yüzde 90'ının sırasıyla 13 ve 10 günde tamamlandığı, toplam biyogaz üretiminin 5413 kJ/kgTS spesifik enerji değerinde yüzde 30 arttığı gözlenmiştir. Buna karşın, tarımsal biyogaz üretim tesisi artık büyokütlesinin anaerobik çürütme prosesinde biyogaz üretiminin bütün spesifik enerji değerlerinde 40 günün sonunda maksimum değere ulaşmadığı ancak gaz üretimindeki artış hızının azalmaya başladığı gözlenmiştir. Bu sonuç, lignin içeriği nedeniyle tarımsal artık biyokütlenin anaerobik olarak çürütülebilirliğinin mümkün olduğunu ancak prosesin daha uzun çürütme sürelerine ihtiyaç duyduğunu göstermiştir.

1. INTRODUCTION

The resource limitation of fossil fuels and the problems arising from their combustion such as the release of toxic compounds and oxides of nitrogen and sulfur into the atmosphere, carbon dioxide emissions that causes global warming, etc. have led to widespread research on new and renewable energy resources (Teleghani and Kia, 2005; Chynoweth et al., 2001).

There are millions of tons of biomass waste, which is highly putrescible, being produced every year form several resources such as forest resources, agricultural resources, municipal solid waste, domestic and industrial wastewater and animal manure. Among the renewable energy resources, waste biomass attracts a special interest because of two main concerns: Firstly, with anaerobic digestion technology it can be converted into vast quantities of biogas, which may directly be used as a comparatively clean energy source. Second, there is a need for effective methods for treatment and disposal of large quantities of municipal, industrial and agricultural organic wastes and anaerobic digestion, which is biological degradation of organic matter in absence of oxygen, is important in controlling organic wastes and producing fertilizer and water for use in agriculture (Chynoweth et al., 2001; Jingura and Matengaifa, 2009).

The major organic constituents of residual biomass after conventional anaerobic digestion on sewage treatment or farm land biogas plants are cellulose, hemicelluloses and lignin bound together in a lignocellulose matrix which are valuable for second generation bio-products such as such as bio-ethanol, biodiesel, methane and bio-hydrogen (Chynoweth and Isaacson, 1987). However, a major drawback to the production and maximum recovery of valuable materials from residual biomass is the structure of lignocellulose, which has evolved to resist degradation and to confer hydrolytic stability and structural robustness due to cross-linking between the polysaccharides (cellulose and hemi-cellulose) and the lignin via ester and ether linkages. Cellulose and hemicelluloe are densely packed by layers of

lignin, which protect them against enzymatic hydrolysis. So it is necessary to break lignin seal to expose cellulose and hemicellulose for enzymatic action.

With the standard anaerobic digestion technologies, only few of this organic matter can be mineralized by further anaerobic digestion. The main goal of any pretreatment would be to alter or remove structural and compositional impediments to hydrolysis and subsequent degradation processes in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products. A substantial increase of biogas production can hence be obtained by applying a proper physical, chemical, thermal, mechanical, or biological pretreatment step, such as ultrasonic treatment, use of (genetically engineered) enzymes and/or treatment (hydrolysis) with sodium hydroxide. The potential of the various pretreatment processes to augment the anaerobic biodegradation rate and produce more biogas is considerable. These methods cause mechanical, physical chemical or biological changes in the biomass in order to achieve the desired products (Godliving and Mtui, 2009).

1.1 Aim of the Thesis

The aim of this study is to investigate the impact of low frequency high-power ultrasound on the performance of anaerobic digestion on the residual biomass of a farm land biogas plant after anaerobic digestion, which contains varying amounts of cellulose, hemi-cellulose and lignin, and to monitor the possible increase in biogas production form lignocellulosic biomass using ultrasonic disintegration.

1.2 Scope of the Thesis

The effects of ultrasound pre-treatment on the characteristics, digestibility and biogas yield of the residual biomass of a farmland biogas plant were evaluated. In second chapter, a backround information on biogas plants, anaerobic digestion mechanisms, sludge disintegration methods are given. Detailed information of ultrasonic diintegration is given olso in this chapter. The methods to evaluate the effects of ultrasound treatment on lignocellulosic biomass are summerized in third chapter and the results of the experiments are given in fourth chapter.

2. BACKGROUND INFORMATION

2.1 What is Biogas?

Biogas is a mixture of gases produced from anaerobic digestion of biogenic material such as biomass, animal manures, sawage sludge, municiple solid waste, and a very important source of renewable methane which is considered as relatively clean fuel since it is lighter in terms of carbon chain length and less amount of carbon dioxide is released into the atmosphere during combustion. Use of biogas technology has advantages over other energy sources because while generating renewable energy, healthy organic waste recycling is achieved and the digestate can be used as bio-fertilizer (Arthur et al., 2011). Organic waste cycling with biogas generation is illustrated in figure 2.1. The complete anaerobic fermentation process is briefly described in section 2.2.

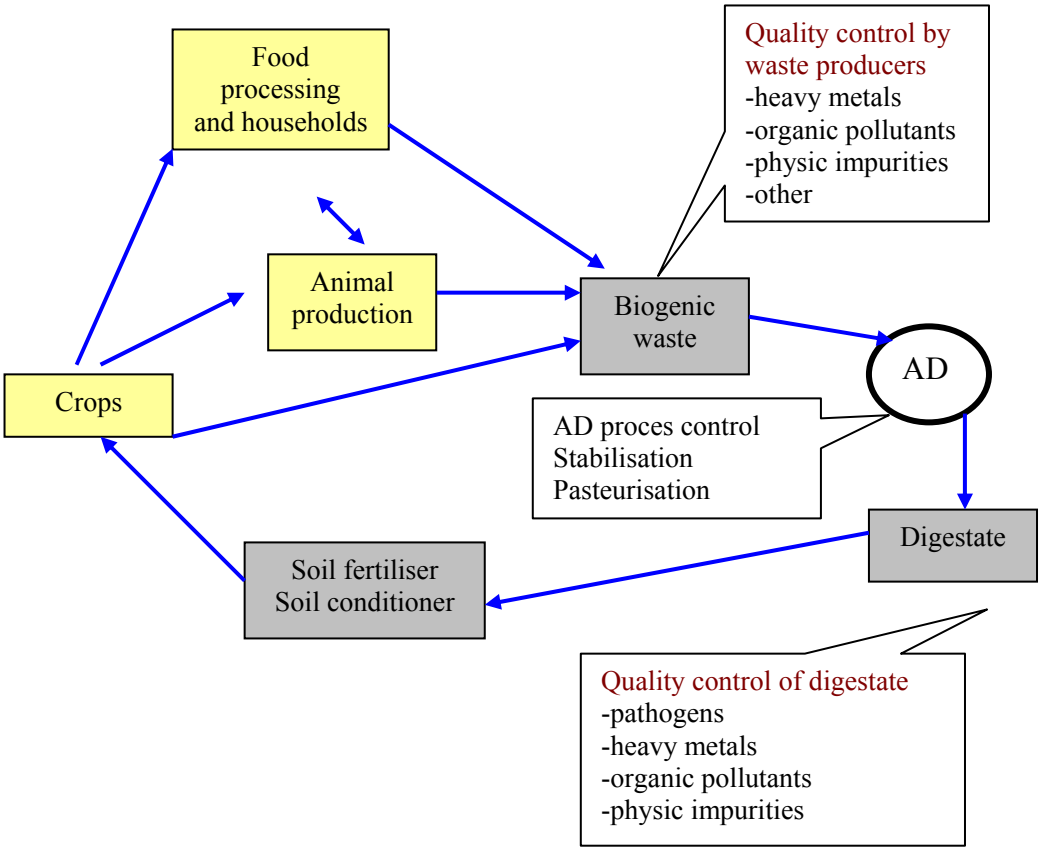

Figure 2.1: Organic waste cycling with biogas production technology

Industrial biogas is produced at sewage treatment plants (sludge fermentation stage), landfills, sites with industrial processing industry and digestion plants for agricultural organic waste (Tippayawong and Thanompongchart, 2010).

The composition of biogas varies depending on the source and the operational conditions used during anaerobic digestion. Typically, raw biogas consists of 40-75 % methane (CH_4), 15-60 % carbon dioxide (CO_2) which is inert in terms of combustion, and trace amounts of other components such as water (H_2O, 5-10%), hydrogen sulfide (H_2S, 0.005-2%), siloxanes (0-0.02%), halogenated hydrocarbons (VOC, < 0.6%), ammonia (NH_3, <1%), oxygen (O2, 0-1%), carbon monoxide (CO, <0.6%) and nitrogen (N_2, 0-2%) (Ryckebosch et al., 2011).

2.1.1 Biogas plants

There are many types of biogas plants in Europe, categorized according to the type of digested substrates, according to the technology applied or according to their size. The biogas plants digesting manure are categorised as agricultural biogas plants, and they usually co-digest manure and other suitable organic residues that are of agricultural origin as well. A common classification of the agricultural biogas plants is:

1- The large scale, joint co-digestion plants and
2- The farm scale plants.

There is not a sharp delimitation between these two categories as elements of technology from one category are also common to one another. The joint biogas plants are generally large scale, with digester capacities ranging from, few hundreds m^3 up to several thousands m^3. The joint biogas plants co-digest animal manure collected from several farms, mixed with suitable organic residues from the food and feed industries and from the overall society (Holm-Nielsen et al., 2009). The farm scale biogas plants co-digest animal manure and slurry from one single farm or, rarely two or three smaller neighboring farms. The applied technology is similar to the joint biogas plants. (Holm-Nielsen et al., 2009).

Fresh animal manure and slurry are collected from the pre-storage tanks at the farms, transported to the biogas plant and mixed with digestible organic wastes. Figure 2.2. illustrates the schema of a farmland biogas plant with co-fermentation.

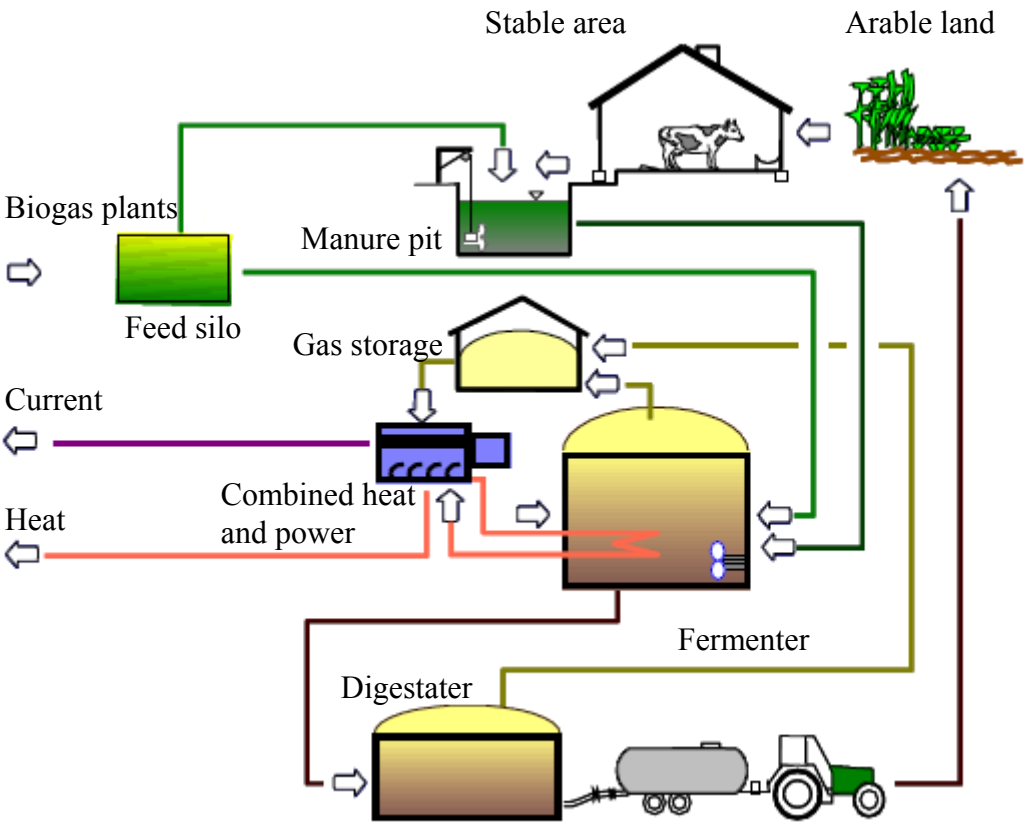

Figure 2.2: A schema of a farmland biogas plant with co-fermentation

2.2 Anaerobic Digestion

Anaerobic Digestion (AD) in a biogas plant is a well proven process in which organic materials are degraded with microbial activity in the absence of oxygen to produce two valuable products - biogas and digestate which are good sources of renewable energy and biofertilizer respectively (Lukehurst, 2010). In this section, the basic principles and parameters of the anaerobic digestion process including the substrate for anaerobic digestion, process description, process parameters and the types of digesters are reviewed.

2.2.1 Substrate for anaerobic digestion

Substrate for anaerobic digestion includes animal manures, agricultural crops, agri-food processing residues, food residues, the organic fraction of household waste, organic fractions of industrial wastes and by-products, sewage sludge, municipal solid waste, etc. The substrate, can be either a single input (e.g. animal manure) or a mixture of two or more feedstock types (co-digestion). Anaerobic micro-organisms

can decompose all kinds of organic materials. Short chain hydrocarbons, such as sugars, are easier to decompose. Longer chain hydrocarbons, such as celluloses and hemicelluloses, are more difficult to decompose and longer digestion time is needed (Themelis and Verma, 2004).

2.2.2 Mechanisms of anaerobic digestion

Anaerobic digestion is a multi-step process which is carried out by a mixed culture of different groups of microorganisms. In the anaerobic digestion process, there are four main stages: Hydrolysis, acidogenesis, acetagenesis and methanogenesis which are carried out by fermentative bacteria, acidogenic bacteria, acetogenic bacteria and methanogenic bacteria respectively. Stages of anaerobic digestion is illustrated in Figure 2.3.

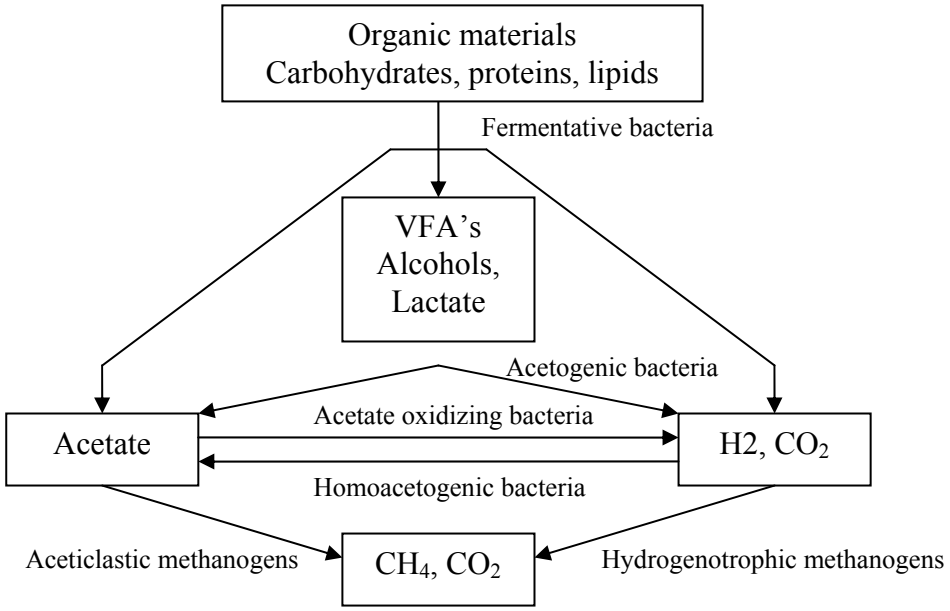

Figure 2.3: Stages in anaerobic digestion process

In hydrolysis step, both insoluble organic materials and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids are degraded in a catalytic reaction controlled by enzymes into soluble organic substances such as amino acids and fatty acids (Appels et al., 2008). Hydrolysis step is generally considered as rate limiting step of AD process (Wang et al. 1999, Tiehm et al., 2001, Bougrier et al., 2005; Aquino and Stuckey, 2008). This limitation leads to long retention times and large tank volumes. To improve this step, several pre-treatment methods are developed by researches which are described in section 2.3.

The components formed during the hydrolysis step are directly available for the acidogenic bacteria which convert these components into simple organic compounds such as volatile fatty acids (VFAs), alcohols and ketones with ammonia (NH_3), CO_2, H2S and other by-products through fermentation. This stage is known as acidogenesis (Appels et al., 2008).

The third stage of AD process is acetogenesis in which the higher organic acids and alcohols produced in acidogenesis step are converted into acetic acid, CO_2 and H_2 by acetagenic bacteria. This conversion is controlled to a large extent by the partial pressure of H_2 in the mixture (Appels et al., 2008). The products of this stage are used in the subsequent process.

The final stage of AD process is methanogenesis produces methane by two groups of methanogenic bacteria: the first group splits acetate into methane and carbon dioxide and the second group uses hydrogen as electron donor and carbon dioxide as electron acceptor to produce methane (Appels et al., 2008). The chemical reactions related to methane production are as follows:

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

Overall, the conversion of organic material to CH_4 involves a close relationship among four types of bacterial populations with the dynamic balance between production and utilization of the intermediate products. This dynamic balance is critical to the overall success of the fermentation and any disturbance would cause accumulation of VFAs and eventually lead to process failure. Further information about the kinetics of AD process can be found in the research of Husain (1998).

2.2.3 Parameters affecting anaerobic digestion process

In anaerobic digestion process, there are various parameters effect the digestibility of sludge and biogas production such as pH, alkalinity, temperature, solids retention time (SRT) and C/N ratio.

2.2.3.1 pH

Each group of micro-organisms has a different optimum pH range. The fermentative microorganisms can function at a pH range of 4.0 to 8.8 whereas methanogenic

bacteria are extremely sensitive to pH with an optimum between 6.5 and 7.2. The volatile fatty acids produced during anaerobic digestion tend to reduce the pH. This reduction is normally countered by the activity of the methanogenic bacteria, which also produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate (Appels et al., 2008).

2.2.3.2 Temperature

Anaerobic digestion can occur in two temperature ranges. The optimum temperature for mesophilic digestion is between 30-40ºC and for termophilic digestion it is between 50-60ºC. Within the temperature range of 40-50ºC which is the upper limit of mesophilic range and lower limit of termophilic range, neither mesophilic nor termophilic microorganisms can grow and function well, therefore, the digestion would be slow and unsatisfactory (Golueke, 1958).

The methanogenic bacteria is sensitive to variations in temperature, so it is important to maintain a stable operating temperature in the digester. Process failure can occur at temperature changes in excess of 1 ºC/day; and changes in temperature of more than 0.6 1ºC/day should be avoided (Turovskiy and Mathai, 2006).

2.2.3.3 Solids retention time

The solids retention time (SRT) is the average time the solids spend in the digester, The subsequent steps of the digestion process are directly related to the SRT. A decrease in the SRT decreases the extent of the reactions and vice versa. Each time sludge is withdrawn, a fraction of the bacterial population is removed thus implying that the cellgrowth must at least compensate the cell removal to ensure steady state and avoid process failure (Appels et al., 2008).

The influence of the retention time on the breakdown efficiency is mostly studied on laboratory scale and theobtained relationship between gas production and retention time in a (semi-) CSTR indicates that (Appels et al., 2008);

1. Retention times shorter than 5 days are insufficient for a stable digestion: VFA concentrations are increasing due to a washout of methanogenic bacteria,
2. VFA concentrations are still relatively high for SRT of 5–8 days: there is an incomplete breakdown of compounds, especially of the lipids,

3. Stable digestion is obtained after 8–10 days: low VFA concentrations, the breakdown of lipids starts, and
4. The breakdown curve stabilises at SRT 410 days; all sludge compounds are significantly reduced. The SRT is a fundamental design and operating parameter for all anaerobic processes.

Retention times less than 5 days are insufficient for digestion because VFA concentrations increase due to a washout of methanogenic bacteria. After 10 days of SRT, sludge compounds are significantly reduced (Appels et al., 2008).

2.2.3.4 Carbon to nitrogen ratio(C/N)

Carbon to nitrogen ratio of input material is also important for anaerobic digestion. It is suggested that the optimum C/N ratio for anaerobic digestion is in the range of 20 to 30 by Parkin and Owen (1986). This value can be achieved by mixing substrates with low and high C/N ratio, such as organic solid waste mixed with sewage sludge or animal manure.

2.3 Sludge Disintegration

Sludge disintegration is destruction of sludge by physical, chemical or biological forces. Depending on the technique and energy input, destruction of sludge floc or disruption of the microorganisms in sludge is achieved. At low energy imput the flocs are destructed and a rapid decrease in particle size takes place. At higher energy input the cell walls of microorganisms are disrupted and organic material in the cells are released. These sludges are suitable as a substrate for a subsequent biological degradation (Muller, 2000). Figure 2.4 illustrates the effect of the energy input on sludge disintegration.

2.3.1 Applications of sludge disintegration

In a wastewater treatment plant, disintegration can be applied at several locations for following purposes:

- Intensification of aerobic and anaerobic sludge stabilization,
- Combating floating sludge,
- Combating foam,

- Production of internal C-source,
- Improving dewatering

The problems caused by scum in the settling tanks and foaming in digesters can be reduced by destruction of filamentous flocs, so the settling properties of bulking sludge can be improved (Muller, 2000).

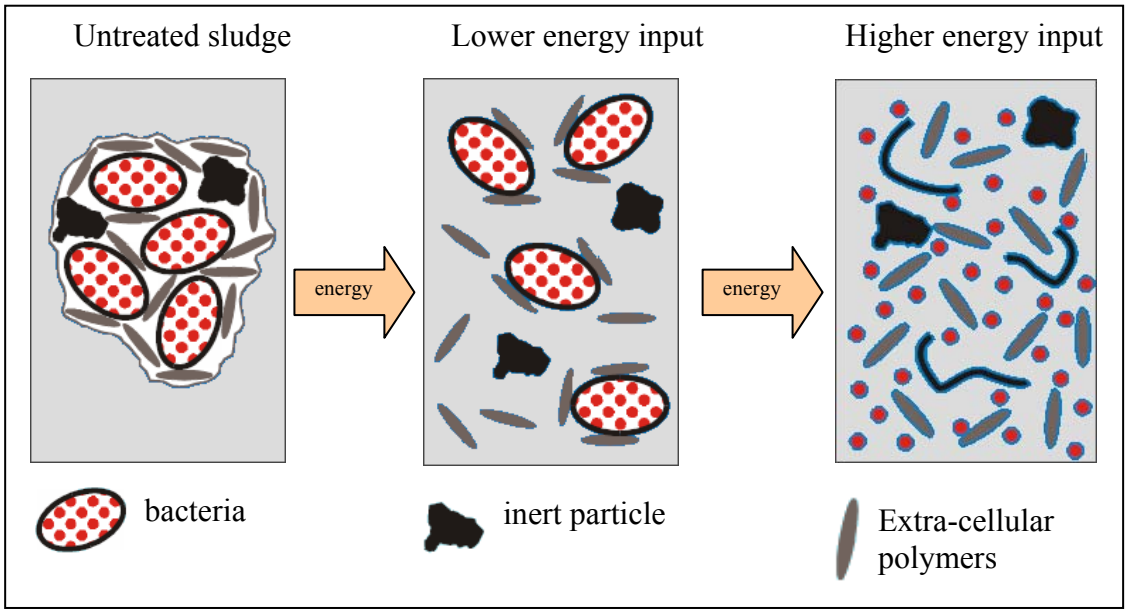

Figure 2.4: Impact of energy input on disintegration of sludge (Lehne et al., 2001)

The disintegrated sludge can be used as a substrate either in aerobic or anaerobic processes as disintegration of microorganisms leads to release of organic substances which are easily accessible to a biological degradation process (Muller, 2000). As a result, degradation degree is improved, amount of excess sludge, degradation time and tank volumes are reduced.

In case of a strong disintegration a large amount of organic solid material is transferred into the liquid phase. The remaining solid sludge particles contain a higher percentage of inorganic substance which results in higher content of dry substance after dewatering (Muller, 2000).

Figure 2.5 illustrates the application points of sludge disintegration in sewage sludge treatment plants.

2.3.2 Sludge disintegration methods

For waste activated sludge minimization and more biogas production than classical anaerobic digestion, several disintegration methods were investigated by researchers.

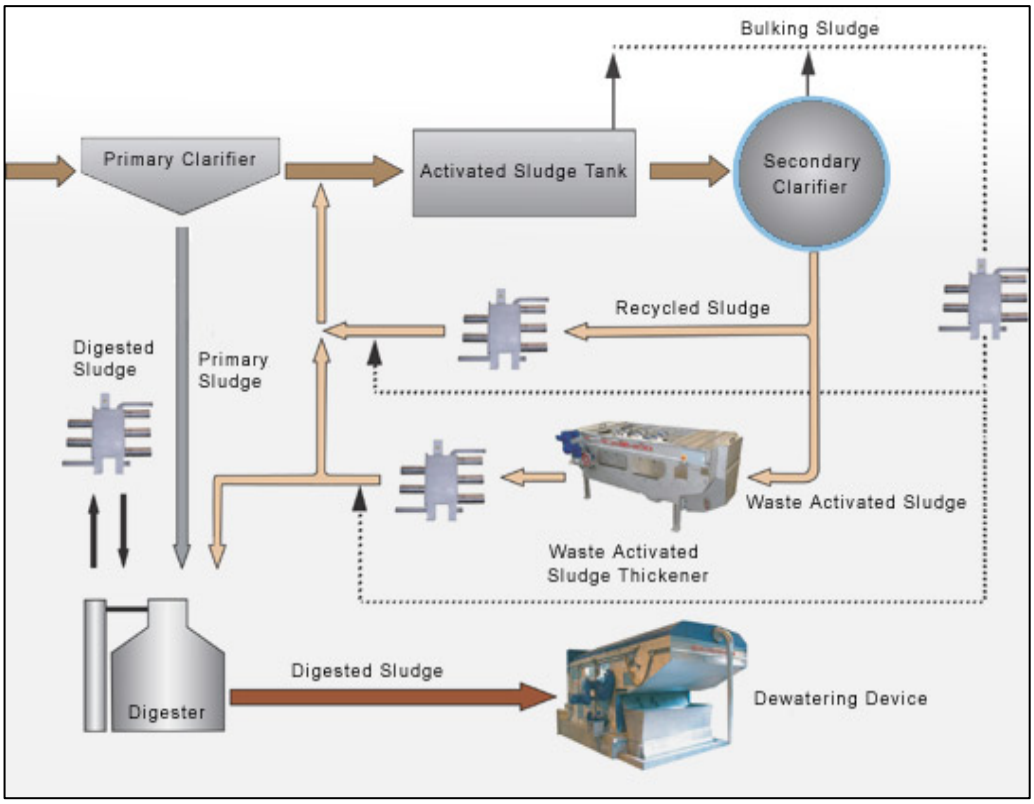

Figure 2.5: Options for sewage sludge disintegration

The methods can be classified as follows (Filibeli and Kaynak, 2006):

- Mechanical disintegration (Stirred ball-mill,High-pressure homogenizer, Ultrasonic Homogenizers, Lysatcentrifuge, Jet Smash Technique, The High Performance Pulse Technique etc.).
- Chemical disintegration (Ozone treatment, Alkaline treatment, Fenton process etc.)
- Thermal disintegration
- Biological disintegration (High temperature sludge stabilization with thermophilic bacteria, Enzymatic lysis).

Although the methods are different, the aim of them is the release of the organic substances inside and outside the cells in the sludge solids into liquid phase.

2.3.2.1 Mechanical disintegration

Mechanical disintegration is disruption of microbial cell walls by shear forces. The energy necessary for disruption of cells is provided as pressure, translational or rotational energy. Mechanical disintegration can be divided into two categories. One

category of mechanical shear typically utilizes violent shearing methods to try to achieve cell lysis and includes such devices as stirred-ball mills, high-pressure homogenizers, blenders and other devices that exert high stresses on the sludge. The violent shearing devices are briefly explained below. The other category of mechanical shear is sonication and could be considered the more refined and less abusive method. (Riedel, 2009). In this study, the effect of sonication on digested biogas plant sludge was investigated, therefore, this method will be explained in details in section 2.4.

Violent shearing devices:

Stirred Ball Mills (SBM) consist of a cylindrical grinding chamber of up to 1 m^3 of volume which is almost completely filled with grinding beads. An agitator forces the beads into a rotational movement. The micro-organisms are disintegrated in between the beads by shear- and pressure-forces. For a continuous operation the beads are held back by a sieve while the suspension can flow through the grinding chamber (Muller, 2000).

High Pressure Homogenizers (HPH) basically consist of a multistep high-pressure-pump and a homogenizing valve. The pump compresses the suspension to pressures up to several hundred bar, realising a flow of up to several cubic meters per hour. The suspension passes through the homogenizing gap while the pressure drops below the vapour pressure of the fluid, and the fluid velocity increases up to 300 m/s. When the occurring cavitation bubbles implode, pressure gradients are induced into the fluid causing temperatures of several hundred degrees Celsius and pressure peaks of $500x10^5$ Pa locally (Muller ,2000).

The Mechanical Jet Smash Technique (MJS) pressurizes the sludge up to $50x10^5$ Pa and then releases the sludge through a nozzle. The accelerated sludge (30 to 100 m/s) smashes onto a plate where the disintegration takes place (Muller, 2000).

The High Performance Pulse Technique (HPP) is an electro-hydraulic method. The sludge is treated by high voltage of up to 10 kV. So a sudden disruption and release of organic substances takes place. The pulse period is only 10 ms, inducing shockwaves in the sludge which lead to disintegration (Muller, 2000).

The Lysat-Centrifugal-Technique (LC) uses a decanter equipped with a disintegration device located at the discharge of the dewatered sludge (Muller, 2000).

2.3.2.2 Chemical disintegration

One of the techniques used in mechanical disintegration is alkaline treatment. In alcaline pre-treatment, the pH of the sludge is increased up to 12 using various alkaline agents such as NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$. This value is generally maintained for 24 hours. Alkaline pretreatment is very effective in terms of solubilizing extracellular polymers (Neyens et al., 2004). Although alkaline pre-treatment is advantageus since it requires low energy, the main disadvantage is the modification of the sludge composition.

Another technique of chemical disintegration is Fenton oxidation process. In Fenton oxidation process, organic substances react with hydrogen peroxide in the presence of inexpensive ferrous sulfate to reduce toxicity and organic load. The oxidation mechanism by Fentons reagent is due to the reactive OH generated in an acidic solution by the catalytic decomposition of hydrogen peroxide. Although the Fenton reaction has been widely studied, there is no agreement on the ratio $[H_2O_2]/[Fe^{2+}]$ that gives the best results. The same occurs with H_2O_2/UV reactions, where an excess of H_2O_2 can act as a hydroxyl scavenger instead of a HO source and which in addition interferes with the determination of the chemical oxygen demand (COD) (Kaynak and Filibeli, 2007; Schrank et al.,2005).

The application of ozone for sludge solubilization has been demonstrated within aerobic and anaerobic sludge digestion systems. The hydrolysis of sludge can be accomplished by exposing it to highly oxidative conditions (ozone) which rupture cell walls releasing soluble COD. Mechanistically, ozone reacts with polysaccharides, proteins, and lipids (which are components of cell membranes), transforming them into smaller molecular-weight compounds. In doing so, the cellular membrane is ruptured, spilling the cell's cytoplasm. (Elliott and Talat, 2007).

Oxidation of ozone takes place through direct ozone reactions and/or indirect reactions of secondary redicals like $OH^{\bullet}$ radicals. Practically, direct and indirect oxidation reactions occur simultaneously, but one type of of these reactions occur dominantly related to some factors like; temperature, pH and properties of matter to be oxidized. The process of sludge ozonation is generally described by the sequential decomposition reactions of floc disintegration, solubilization, and the subsequent oxidation of the released organics into carbon dioxide (mineralization) (Ahn et al., 2002; Lee et al., 2005). Basically, it is supposed that one-oxygen atom of O3 reacts

with the oxidant. This means that 48 g of ozone can stoichiometrically decompose 16 g of COD (mineralization). However, the detected mineralization is generally lower than this value. Lee et al. (2005) reported that with 0.05 g O3/g total suspended solid (TSS) ozone contact, raw sludge was significantly transformed and resulted in 8% mineralization, 22% solubilization and 70% residuals based on chemical oxygen demand (COD).

2.3.2.3 Thermal disintegration

Thermal sludge pre-treatment was originally used to improve the dewaterability of the sludge. Heat treatment results in the breakdown of the gel structure of the sludge and the release of intracellular bound water. The corresponding release of intracellular compounds was seen as an important drawback, but is now becoming interesting as a pre-treatment prior to anaerobic digestion and as a means of producing internal carbon sources for nutrient removal.

Most investigations involving thermal pretreatment have used exposure temperatures ranging between 150 and 200 ºC. Jolis et al. (2004) found that the thermal hydrolysis of primary and secondary municipal sludges at a very high temperature of 270 ºC (for 25 min) prior to digestion in a temperaturephased anaerobic digester (TPAD) allowed higher organic loading and increased VS destruction and gas production. The digested sludge was classified as ‘‘Class A’’ biosolids. The costeffectiveness of this intense pretreatment was not analyzed. Ferrer et al. (2006) found low-temperature thermal pretreatment to be advantageous, as compared with high-temperature pretreatment, in terms of gas production from thermophilic anaerobic digestion. In their study, the municipal sludge was conditioned at 110–134 ºC (for 20–90 min) and at 70 ºC (for 9–72 h) before thermophilic anaerobic digestion. Additional samples of sludge were also conditioned with ultrasound (300W at 20 kHz) and microwaves (800W at 2450 MHz) to provide a comparison with thermal treatment. Though all pretreatments increased soluble organic content of the sludge, only the low temperature (70 ºC) treatment showed a positive effect on biogas production.

2.3.2.4 Biological disintegration

The biochemical sludge disintegration is based on enzyme activity that are either produced within the system (autolysis) or externally. The enzymatic lysis cracks the

compounds of the cell wall by an enzyme catalysed reaction. Even Gram-positive bacteria with a high strength cell wall can be disintegrated using enzymes. Autolytic processes can be used at ambient temperatures or external enzymes can be added, resulting in additional treatment costs. Enzymatic lysis is of interest in combination with mechanical disintegration as well, because enzymes are also located in the intracellular liquid. They can cause a further disintegration of the cells after a mechanical disintegration by autolysis (Dohanyos et al., 1997). This process is of interest in combination with mechanical disintegration as well, because enzymes are also located in the intracellular liquid (Thomas et al., 1993).

Biological hydrolysis can be considered as a partial anaerobic sludge digestion. In conventional anaerobic digestion processes, acidogens and acetogens first solubilise and hydrolyse sludge microbes prior to the actual conversion to methane by the methanogens. By controlling the hydraulic retention time and temperature, it is possible to confine the anaerobic digestion of sludge to the acidogenic and acetogenic phase (hydrolysis and fermentation process) and take advantage of the soluble organics produced. In practice, biological hydrolysis is applied to primary sludge in order to produce an in-situ carbon source for nutrient removal (Weemaes and Verstraete, 1998).

2.4 Ultrasonic Disintegration

Ultrasound is a sound wave at a frequency above the normal hearing range of humans (>20 kHz) (Khanal et al., 2007). Figure 2.6 shows the range of sound frequency.

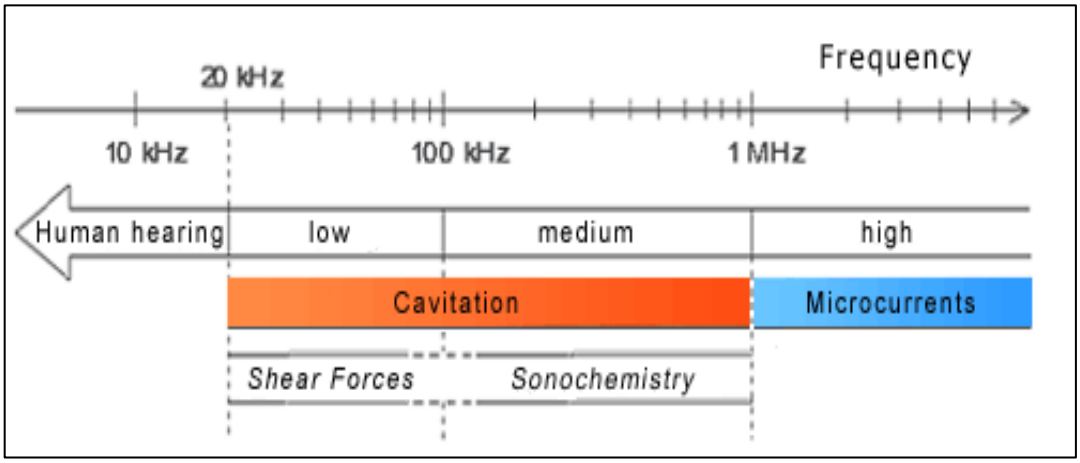

Figure 2.6: The range of sound frequency

Ultrasonic disintegration is a well-known method for the break-up of microbial cells to extract intracellular material available for subsequent degradation to CH_4 and CO_2 in anaerobic digestion (Tiehm et al., 2001)

Ultrasound equipment consists of three major components. A transducer (converter) which converts the electrical or mechanical energy into sound waves, a booster which is a mechanical amplifier that increases the amplitude generated by transducer and the sonotrode (horn) which transmits the ultrasound waves into the liquid (Pilli et al., 2011; Khanal et al., 2007). Figure 2.7 shows the ultrasonic equipment and ultrasonic reactor.

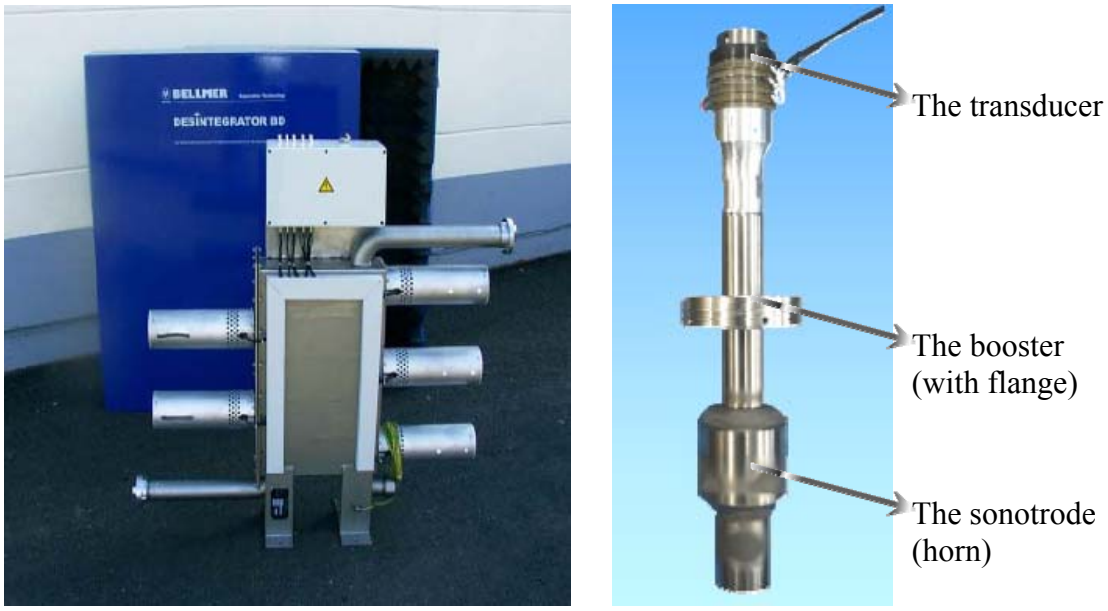

Figure 2.7: Ultrasonic equipment

When applied to water, ultrasound generates compressions and rarefactions, the compression cycles exert a positive pressure on the liquid by pushing the molecules together and the rarefactioncycle exerts a negative pressure by pulling the molecules from one another. Because of this excessively large negative pressure, microbubbles (cavitation bubbles) are formed in the rarefaction regions. These cavitation bubbles grow and then violently collapse within a few microseconds when they reach a critical diameter. Figure 2.8 illustrates the growth and critical size of microbubbles.

Collapse of the microbubbles produces extremely high local temperature (5000ºC) and pressure (500 atm) on liquid-gas interface, turbulence and high shearing phenomena in the liquid phase (Flint and Suslick, 1991; Pilli et al., 2011). The extreme local conditions can lead to the thermal destruction of compounds present in

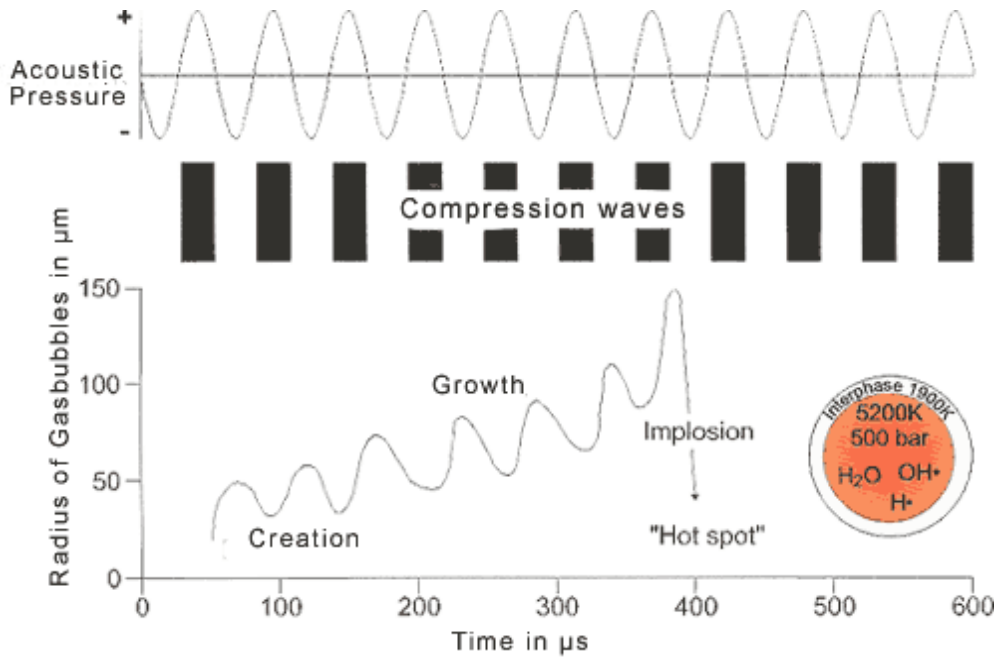

Figure 2.8: The growth and critical size of microbubbles

cavitation bubbles and to the generation of very reactive radicals and hdrogen peroxide Thus, sonication is combination of different phenomena (Wang et. al., 2005);

- Hydro-mechanical shear forces;
- Oxidizing effect of $OH^{\bullet}$, $H^{\bullet}$, $N^{\bullet}$ and $O^{\bullet}$ produced under the ultrasonic radiation;
- Thermal decomposition of volatile hydrophobic substances in the sludge;
- Increase of temperature during ultrasonic activated sludge disintegration.

The sludge disintegration is better at lower frequency range of 20-40 kHz due to strong hydrodynamic shear resulting from cavitation effects at low frequency (Tiehm et al., 2001; Khanal et al., 2007). Wang et al. (2005) recently explored the mechanism of ultrasonic disintegration of WAS at a frequency of 20 kHz.

2.4.1 Effects of ultrasound treatment on the characteristics of sludge

During ultrasound treatment physical, chemical and biological properties of the sludge are changed. Therefore, the degree of sludge disintegration is evaluated based on the changes in physical (particle size distribution, turbidity, settlability, mass composition and microscopic examination), chemical (increase in SCOD, protein concentration, polysaccharide content of the supernatant, nitrate nitrogen and release of NH3) and biological (heterotrophic count and specific oxygen uptake rate) properties (Pilli et al., 2011).

2.4.1.1 Physical evaluation

Particle size: Ultrasound pretreatment is very effective in reducing the particle size of sludge and the efficiency of size reduction is depend upon the sonication duration ultrasonication density, sonication power, sludge volume and sludge characteristics (Pilli et al., 2011). Tiehm et al. (1997) applied ultrasound treatment to a sludge sample at a frequency of 31 kHz and power input of 3.6 W and reported that the d50 of sludge particles decreased from 165 μm to 135 and 85 μm during 29.5 and 96 s of ultrasonic treatment respectively. Bougrier et al. (2005) evaluated the effect of ultrasonic treatment on particle size distribution after different specific energy inputs at 20 kHz frequency and supplied power input of 225 W. The cut diameter, d50 of sludge particles decreased with increase in specific energy input. The d50 at specific energy inputs of 0 (control), 660, 1,350, 6,950 and 14,550 kJ/ kgTS were 32, 19.6, 18.5, 17.6, and 12.7 μm, respectively. After a certain point, the particle size starts to increase with increasing sonication time because of re-flocculation of the particles due to the more release of intracellular polymers. Gonze et al. (2003) observed a reduction trend up to 10 min. of sonication, but after this point the particle size has increased gradually. Total solids concentration also effects particle size reduction. Akın et al. (2006) studied the effect of sonication on particle size at different total solids concentration in sludge. The size reduction was more for the lower TS sludge, and a similar degree of reduction required more ultrasonication density in the higher TS content.

Dewaterability of sludge: Ultrasonication with lower energy input and less sonication time increases sludge dewaterability, but degree of disintegration is not sufficient as there is no cell lysis. The sludge dewaterability is optimum when the degree of disintegration is between 2% and 5%. Below disintegration degree of 2%, the change in sludge floc structure is very limited and above 5% the bound water content increases (Huan et al., 2009). Chu et al. (2001) observed that the dewaterability of sludge decreased gradually with an increase in sonication time, because the greater increase in the amount of the small particles formed after sonication created a larger surface area for water. The dewaterability of the sludge becomes worse with increase in ultrasonication intensity due to cell lysis and release of biopolymers from extracellular polymeric substances (EPS) and bacteria in the aqueous phase (Wang et al., 2005).

Settleability of sludge: The settlability of sludge changes with increase in specific energy. Feng et al. (2009) have reported that the optimum specific energy for improving the WAS settleability is 1000 kj/kg TS. When the specific energy is greater than 5000 kj/kg TS, the settleability of WAS decreases due to complete breakdown of flocs and increase of extracellular polymeric substances concentration in the sample.

Floc disintegration and Cell lysis: Ultrasonic treatment breaks down the sludge flocs and disrupts cell wall of the micro-organisms. Complete breakdown of the flocs and cell wall occur at longer sonication times. Khanal et al. (2006) observed that at 2 min. of sonication, the structural integrity of the flocs as well as filaments are significantly disrupted without significant destruction of bacterial cells. At 10 min. of sonication the flocs are complately disintegrated and filament-like structures with a few scattered bacterial cells, and at a 30 min. of sonication, more or less the complete break-up of cell walls occurred.

2.4.1.2 Chemical evaluation

Ultrasonic Treatment increases the soluble COD of sludge due to solubilization of solid phase matter and increase in the concentration of organic matter. SCOD can be used as a parameter to determine the degree of disintegration. Muller (2000) proposed following equation which is modified from Kunz and Wagner's (1994) equation to evaluate the degree of disintegration:

$$DD = [(COD_u - COD_0) / (COD_{NaOH} - COD_0)] \times 100$$

where;

COD_u = COD concentration of sludge after disintegration,

COD_0 = Initial COD concentration of raw sludge sample,

COD_{NaOH} = The maximal value of COD, able to be obtained in the supernatant after alkaline hydrolysis of the sludge

Although most of the researchers used the change in soluble COD concentration to determine sludge disintegration efficiency, comparison of the results is very difficult, because sludge disintegration could vary depending on sludge type, TS content, power supply, ultrasonic frequency, ultrasonic density, temperature, sonication time, etc. (Pilli et al., 2011).

Ultrasound treatment also increases organic nitrogen and ammonia concentration in sludge samples. Khanal et al. (2006) studied the release of ammonia-N concentration at different TS contents and specific energy inputs (kJ/gTS) during ultrasonic disintegration of WAS and showed that the release of ammonia-N concentration increased with increase in specific energy inputs and TS contents. The ammonia-N concentration reached a fairly constant level at specific energy input of 20 kWs/gTS for 2.0, 2.5 and 3% TS content. The ammonia nitrogen values were 90, 100 and 200 mg/L respectively. For 1.5% TS content the constant level of ammonia-N (60 mg/L) was reached at specific energy input of 10 kWs/gTS. During sonication, bacterial cells are disintegrated releasing intracellular organic nitrogen into the aqueous phase, which is subsequently hydrolyzed to ammonia. This results in an increase in ammonia nitrogen in the aqueous phase. It is important to point out that the disintegration of organic nitrogen from nonbiological debris could also contribute to the release of ammonia nitrogen.

2.4.1.3 Biological evaluation

Ultrasonic treatment results in disruption of the flocs and break-up of the cell wall of bacteria. Since the WAS mainly consists of aerobic and facultative bacteria, oxygen uptake rate (OUR) can be measured as an indicator of bioactivity of waste activated sludge. OUR measured before and after sonication can be used to determine the effect of ultrasound treatment. Rai et al. (2004) proposed following equation to calculate the degree of disintegration based on OUR measurements:

$DD_{OUR} = [1- OUR_u/OUR_0]. 100$

where;

OUR_u = Oxygen uptake rate of sonicated sludge,

OUR_0 = Initial oxygen uptae rate of raw sludge sample,

The DD_{OUR} have been observed to increase rapidly with increase in specific energy input up to 40 kj/g TS. After this point the increasing rate of DD_{OUR} slowed down (Rai et al., 2004). At a specific energy input of 8 kJ/gTS, the DD_{OUR} was found to be negative which means that the OUR of sonicated sludge was higher than that of the unsonicated, because at low energy input, the microbial cells were not disrupted and the flocs were simply deagglomerated into individual microbial cells, which eventually participated in the biological activity. Since the OUR measures the real

biological activity, DD_{OUR} determination based on OUR measurement could be a very useful tool for field application to assess the ultrasonic disintegration of sludge.

As waste activated sludge mainly consists of heterotrophic bacteria, the measure of their survival during ultrasonic treatment could also be used to determine the efficacy of ultrasonic disintegration. Chu et al. (2001) evaluated the survival ratio using the heterotophic plate count and oxygen uptake rate measurements of the sonicated sludge samples. The survival ratio was 44% for heterotrophic bacteria at a sonication density of 0.33 W/ml during 120 min of sonication.

2.4.2 Literature review

Neis et al. (2000) studied the improvement of anaerobic digestion by ultrasonic disintegration using a pilot- scale plant which consists of a 3.6 kW ultrasound reactor and five stirred tank fermenters. The pilotscale reactor was developed for operation at a low frequency of 31 kHz. The average waste activated sludge (WAS) retention time was 16 days. The dry solids (DS) content of the thickened WAS varied between 0.7 and 2.6% and the volatile solids (VS) concentration was 78%. Two control fermenters were operated with untreated sludge at sludge retention times (SRT) of 16 and 8 days. Three fermenters were fed with ultrasonically treated sludge at SRT of 16, 8 and 4 days. The enhanced degradation rates resulted in a significant increase of biogas production. Specific biogas yields ranged between 520 and 730 L/kg VS degraded. The methane concentration of the biogas varied between 67 to 72%.

That study demonstrates that ultrasonic cell disintegration is a suitable method to overcome the slow biological sludge hydrolysis. Consequently the fermentation rate was significantly increased. Higher removal rates allow shorter sludge residence times. A decrease in sludge residence time from 16 to 4 days didn't show any loss in degradation efficiency. An increased production of biogas was also observed. According to this study, ultrasound treatment of waste activated sludge is a reliable method to reduce the solids retention times in the anaerobic digesters and necessary volume of digesters. Higher removal rates lead to higher degree of volatile solids degradation.

Tiehm et al. (1997) applied low frequency ultrasound (3.6 kW, 31 kHz, 64 s) to sludge and showed that ultrasonic disintegration can release the organic substances into the sludge. In this sstudy, the soluble chemical oxygen demand (SCOD) in the

supernatant increased from 630 to 2270 mg/L. Furthermore, the digestion time reduced from 22 days to 8 days.

Bougrier et al. (2005) studied solubilization of waste activated sludge by ultrasonic treatment. Different ultrasonic energy supplies ranged from 0 to 15,000 kJ/kg TS were applied to the activated sludge in their study with a constant operating frequency of 20 kHz and a constant supplied power of about 225W. The results of that study showed that COD, organic matter, biogas production and nitrogen solubilisation increased with supplied energy. The ultrasonic process led to floc size reduction and cells lysis. For specific supplied energy lower than 1000 kJ/kg TS, energy was used in order to reduce flocs size. Then, supplementary energy was used to break flocs or cells. That permitted the release of organic substances into the liquid phase. Organic substances were more available, so biodegradability was improved. In terms of biogas production, it did not seem interesting to have a supplied energy higher than 7000 kJ/kg TS. Indeed, when the supplied energy was higher than 7000 kJ/kg TS, biogas generation was constant and solubilisation was less marked.

Muller et al. (2005) used mesophilic reactors (38 L with 15-day retention times) to determine the impact of ultrasonic pretreatment and its point of application on treatment performance. Various scenarios were evaluated; including pretreatment of WAS before entering the anaerobic reactor and that of the anaerobic sludge recycling within the anaerobic digesters. Although the test results showed only a small difference for the two cases, a correlation was found to exist between gas production and energy applied. The authors elaborated further that, upon scale-up, one should expect the recycle sonication system to outperform a WAS pretreatment system. This would come as the majority of solids in the recycle line are refractory compounds which would be specifically disintegrated by sonication. Contrary to this, the pretreatment of WAS exposes both the readily degradable substrate as well as the refractory compounds, thus requiring more energy. The gas production was improved by 17%, with a 6.2% increase in total solids destruction. This enhanced degradation coincided with an increase in nitrogen in the liquid phase, suggesting that the protein component of the sludge flocs had been degraded.

One of the full-scale trials to demonstrate the effectiveness of sonication pretreatment was performed by Xie et al. (2005). The authors reported on a trial at a municipal treatment facility in Singapore that uses egg-shaped anaerobic digesters to

treat its primary and secondary sludges. Two full-scale digesters (4500m3 volume), an experimental and a control digester, were used for the trial. The sludge flow rate was 200m3/d. The experimental line was fed sludge which had passed through an ultrasound chamber, while the reference line received sludge without pretreatment. The ultrasound facility consisted of a 20 kHz generator and a stack of five donut-shaped horns which produced a central holding capacity of 3.5 L. The exposure time to sonication was estimated to be 3.5 s. The solids retention time (SRT) in the digesterswas approximately 30 days. Over a 6-month data collection period, the experimental system consistently produced a minimum of 200m3/d more gas than the reference system. Marginal decrease in total suspended solids (TSS) and volatile suspended solids (VSS) was reported after sonication. The authors postulated that there would be a net energy gain of 3.6 times if onlyWAS was treated.

Another full-scale trial in Avonmouth, UK, where one of the six mesophilic digesters (2700m3, 12 day HRT) received WAS pretreated with Sonix technology was performed by Hogan et al., 2004. Primary sludge, which is relatively easy to digest, remained untreated. Sonication pretreatment of the WAS resulted in three times more sludge being assimilated in the anaerobic digester with a 20–30% increase in gas production. A similar 5-month trial was performed at the WWTP in Orange County, US, with a 50% increase in gas production (Hogan et al., 2004).

Not all studies confirmed an enhancement of VS reduction and higher biogas production with ultrasound pretreatment. Sandino et al. (2005) reported an insignificant increase in VS destruction based on a 7-month pilot trial using 18 L mesophilic digesters. Pretreatment by sonication only resulted in a faster rate of reaction but the VS reduction endpoint remained the same. This finding suggests that smaller anaerobic reactors could be designed with sonication pretreatment, while achieving the same rate of VS reduction and gas production.

3. MATERIALS AND METHODS

In this section materials, methods and measurement parameters utilized during the laboratory experiments were outlined.

3.1 Sampling

The digestate used in this study was obtained from the secondary digester of farmland biogas plant Wegener, Bispingen Germany. The sample was stored in the fridge for maximum 1 day before the start up of the tests to avoid significant microbial degradation. Innoculum used in Biomethane Potential Test was obtained from the primary digester of the same plant.

Wegener farming biogas plant uses anaerobic digestion technology to ferment farm products to produce biogas for electricity and heating, and digestate is used as fertilizer. The main feed of the plant is maize mixed with liquid manure. The details about plant, material composition and biogas production can be seen in Table 1.

Table 3.1: Description of Wegener farmland biogas plant

Characteristics	Description
Feed of the plant	6% Corn (82% VS) 63% Maize (32% VS) 5% Manure (40% VS) 26% Liquid manure (cattle)(10% VS)
Total amount of substrate	48.3 t fresh substrate/ d 48.3 t dry solid / d
Production of biogas	8.667 m3/d
Generation of electricity	16.434 kWh/d
Conveyance	The contribution of the feed is aided by recirculation of the digester content
Primary Digester	2 tanks each 1.300 m3
Secondary Digester	2 tanks each 1.300 m3 1 tank 2.300 m3
Hydraulic retention time	>100 d
Electrical Power	1100 kW

3.2 Methods Used in Experimental Analysis

3.2.1 Total solids and volatile solids

The AWWA standard method (APHA, AWWA and WEF, 2005) was used to determine total solids (TS) and volatile solids (VS). To measure the TS, the sample was heated up to 105°C in order to remove all water content in the sample. After finishing TS measurent, the heating of the sample up to 550°C over 2 hours was continued, so all organic matter was burned. The weight difference after heating up 105°C and 550°C reflects VS content.

The TS and VS was calculated for sample, innoculum and all of the reactors in BMP test system before and after the anaerobic digestion.

The equipment used to determine TS and VS are shown in Figure 3.1.

3.2.2 Chemical oxygen demand

The amount of colloidal and soluble COD was measured for untreated and sonicated samples as well as for all the reactors before and after anaerobic digestion. Samples were centrifuged at 18,000 rpm for 10 minutes at room temperature and supernatant was passed through 5 µm and 0.45 µm cellulose filters. Required amount of filtrates were added to the Lange cuvette and heated to 148°C for 2 hours. The organic components in the sludge sample react with potassium dichromate. Using a spectrophotometer, the amount of chromate produced is measured colourimetrically.

		
Analytic Balance: Sartorius MC1 AC 210S	Electric Oven: Memmert (105 ºC)	Muffle Furnace: Heraeus M 104 (550 ºC)

Figure 3.1: Equipment used to determine VS and TS

Measured COD concentrations of filtrates from 0.45 µm filter were considered as soluble COD. The difference between the COD concentrations of filtrates from 5 µm and 0.45 µm filters were considered as colloidal COD. The equipment used to determine COD are shown in Figure 3.2.

	
Centrifuge: Survall Super T 21	Photometer: LANGE CADAS 200

Figure 3.2: Equipment used to determine COD

3.2.3 Total organic carbon and total nitrogen

TOC and TN concentrations were analysed for each sample using spectrophotometer. The equipment used in measurement is shown in Figure 3.3.


Analyser: Analytic Jena Multi N/C 3000

Figure 3.3: Equipment used to determine TOC and TN

3.2.4 Ammonia nitrogen

NH_4-N concentrations were determined for each sample using Lange cuvettes and Lange photometer. Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol blue.

3.2.5 Protein

The protein concentrations of the untreated and treated samples and the mixtures in the reactors were measured using Lowry method. For protein measurements the samples were centrifuged at 18000 rpm for 10 minutes and the supernatants were filtered from 5 µm and 0.45 µm cellulose filter papers. Before the measurements, the filtrates were diluted to 1/50. The equipment used to determine protein concentrations is shown in Figure 3.4.

Spectrophotometer:
JASCO V-550

Figure 3.4: Equipment used to determine protein concentration

3.2.6 Ultrasound treatment

Laboratory scale sonication unit at Ultrawaves GmbH laboratory was used to conduct ultrasonic treatment. The sonication unit is a reactor equipped with a sonotrode as given in Figure 3.5. The maximum power of the sonotrode is 1 kW. The sample was treated for 1, 3, 6 and 10 minutes to see the effect of sonication time on the solubilisation of the sludge and biogas production. Sonication was applied at a

frequency of 21 kHz. 700 ml of digestate is used for the treatment. The digestate was poured into a beaker and placed on a stand in the reactor. Complete emersion of the sonotrode was ensured by adjusting the stand. An energy counter was attached to the power source and the time was set on a stop watch. The reactor was switched on and the stop watch was activated simultaneously. The power was recorded in every 15 seconds to aid in calculating the energy input. The soluble COD was determined for each treatment time immediately after the process. Treated samples are immediately placed in the biomethane potential test.

Generator: Sonotronic Nagel

Horn Sonotrode inside Protective Case

Energy Monitor: ELV EM600

Figure 3.5: Equipment used in ultrasonic treatment

3.2.7 Biomethane potential test

In order to see the effect of ultrasonic treatment on the biogas production potential of the digestate, BMP test was performed. An automatic BMP test system was used for the test. The reactors were prapered with untreated and 1, 3, 6, and 10 minutes sonicated samples with 1:1 innoculum/substrate ratio based on VS contents. Additionally, inoculum with microcrystalline celluleose reactors with 3:1 inoculum/ cellulose ratio based on VS contents were prepared for comparison. Blank reactors containing the same amount of inoculum as other reactors were prepared in order to be able to withdraw the amount of gas produced from the inoculum from the volume produced with both inoculum and substrate. The same volume of inoculum supplied to the reactors and substrate volumes adjusted accordingly. A total volume of 400 ml for each mixture were added to the 600 ml flasks to avoid problems if foaming occurs. Distilled water was added to the reactors containing only innoculum to

provide the same volume for all reactors. The flaskes are equipped with a stirring stick connected to a motor to mix the samples in a desired frequency.

All of the reactors were placed into a water bath with temperature adjustment. The water temperature was adjusted to 37º C to provide mezophilic conditions for anaerobic digestion.

NaOH bottle were prepared for CO_2 fixation. For each reactor, one bottle containing 80 ml of 3M NaOH solution and 0.5 ml of Thymolphthalein pH-indicator and the bottles were stirred continuously with a magnetic stirrer.

The reactors were connected to the NaOH bottles, and NaOH bottles were connected to the flow cells which measure the produced gas continuously. The measured gas volumes were transferred to the data logger. The equipment used for biogas measurement can be seen in Figure 3.6.

ABMPT System: Bioprocess Control

Figure 3.6: Equipment used to determine biogas measurement

3.3 Experimental Set-Up

Including the blank and control samples, 7 mixtures were prepared in doubles for anaerobic digestion step. The operational parameters of each reactor is listed in Table 3.2.

The TS and VS values of inoculum + cellulose mixture was not included to this table because it was observed that there was significant loss of VS during the drying step of the standard TS measurement method so TS of cellulose solution can not be

measured correctly. Because the cellulose amount added as substrate was certain, the theoretical VS value of 7.55% for that sample was used for the related calculations.

The experimental set up of this study is illustrated in Figure 3.7.

Table 3.2: Operational parameters for each anaerobic digestion reactor

Mixture	Total Volume (ml)	TS (%)	VS (%)	pH	$VS_{substrate}/VS_{inoculum}$
Innoculum + Deionized Water	400	2.52	2.88	7,55	-
Innoculum + Cellulose	400	-	-	7,65	0,33
Innoculum + Untreated Substrate	400	9.74	6.80	7,86	1
Innoculum + 1 min. Sonicated Substrate	400	9.90	6.51	7,89	1
Innoculum + 3 min. Sonicated Substrate	400	9.57	5.94	7,85	1
Innoculum + 6 min. Sonicated Substrate	400	9.36	6.05	7,86	1
Innoculum + 10 min. Sonicated Substrate	400	8.49	5.56	7,87	1

3.4 Calculations

3.4.1 Disintegration degree

DD parameter is calculated as following equation:

$$DD = [(COD_u - COD_0) / (COD_{max} - COD_0)]. 100$$

where;

COD_u = Soluble COD concentration of sludge centrate after ultrasonic pre-treatment,

COD_0 = Soluble COD concentration of raw sludge centrate,

COD_{max} = COD concentration of sludge centrate after treatment with NaOH

However, it should be noted that a chemicel disintegration with NaOH was not done for this sample as effort to clarify the procedure was not productive. Instead, the COD for the NaOH treated sample was calculated according to the following equation using an empirical factor 550 as recommended by Ultrawaves GmbH:

$$COD_{NaOH} = VS \times 550$$

Where:

CODNaOH : COD of a reference sample that is hydrolysed in NaOH (mg/L)

VS : Volatile solids of untreated sample (g)

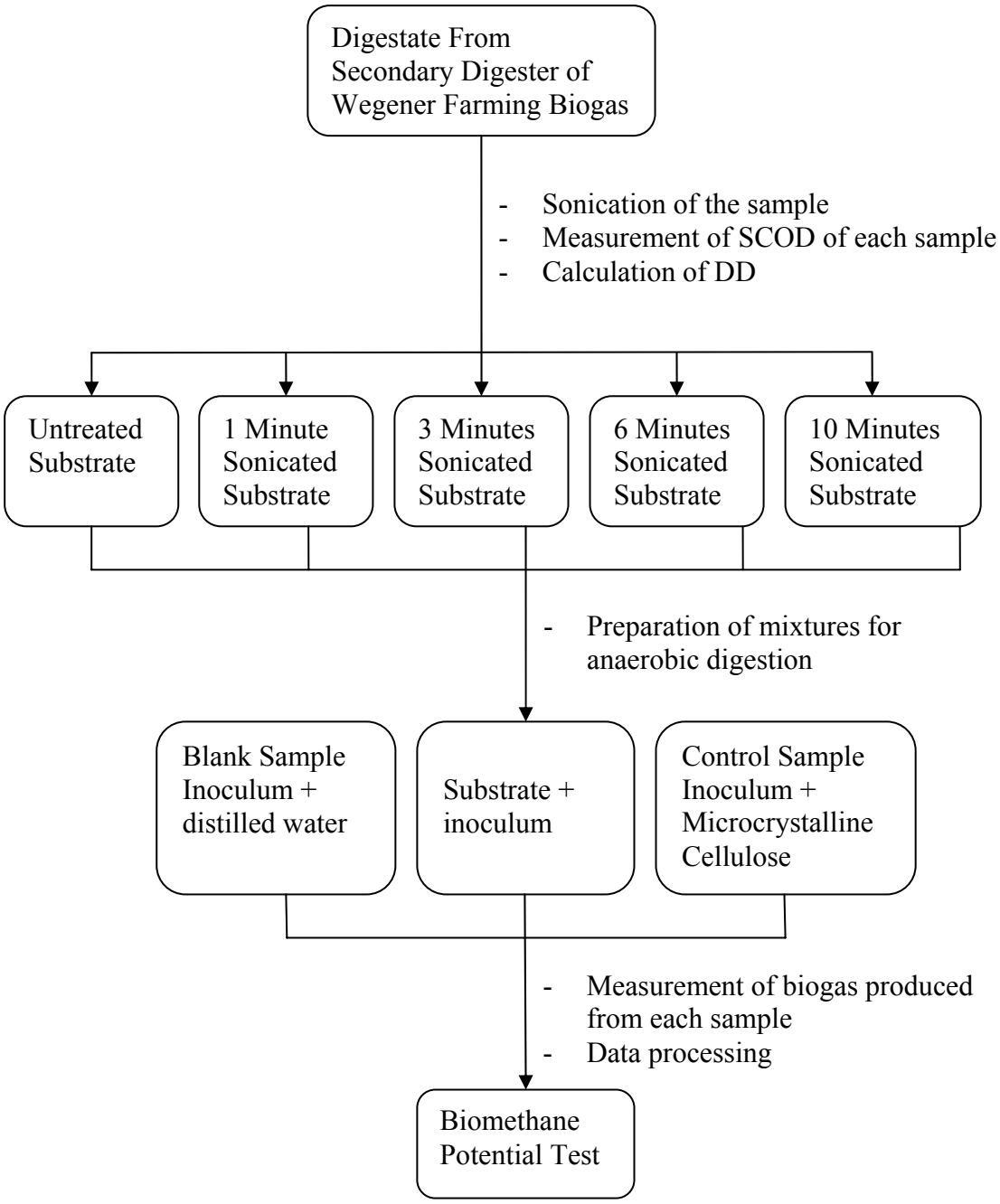

Figure 3.7: Experimental set-up

3.4.2 Specific energy

The power input, TS content, sonication time and volume of sludge to be sonicated are the important factors affecting the ultrasonic disintegration. These parameters can be lumped together into a single parameter, commonly known as "specific energy input." The specific energy input was calculated using the following equation:

$SE = (P \times t) / (V \times TS)$

Where,

SE : Specific energy input (kJ/kgTS)

P : Power inputs (W)

t : Sonication time (second)

V : Volume of sludge used for sonication (L)

TS : Total solids (g/l)

The ultrasonic pre-treatment conditions and calculated specific energy values for each sample is listed in Table 3.3 below. Because the power was not adjustable, it decreases as the sonication time increases.

Table 3.3: Ultrasonic treatment conditions for each sample

Sample Volume (ml)	Total Solids (%)	Sonication Time (min)	Power (W)	Specific Energy (kJ/kgTS)
700	7.98	1	241	259
700	7.98	3	235	758
700	7.98	6	230	1484
700	7.98	10	217	2334

4. RESULTS AND DISCUSSION

4.1 Caracteristics of Substrate

In this study, ultrasonic treatment was applied with various durations to digestate from a farming biogas plant to investigate the effect of ultrasonic treatment on biogas production. The digestate was taken from the secondary digester and inoculum was taken from main digester of the plant. The caracteristics of digestate and inoculum are given in Table 4.1.

Table 4.1: Characteristics of Substrate and Inoculum

Parameter	Inoculum	Substrate
TS (%)	8,82	7.98
VS (%)	5.67	5.52
VS/TS (%)	64.3	69.2
SCOD, mg/L	9217	7865
TOC, mg/L	6870	7730
TN, mg/L	2935	3090
pH	7.55	7.86

As it can be seen in the table, %VS/TS of digestate form secondary digester was slightly higher than %VS/TS of the inoculum, although the other way round was expected. Insufficient mixing in the main digester could have caused the inorganic solids to settle and decrease the %TS in secondary digester so that percentage of VS had increased. Inhomogeneous composition of the sludge could also be another reason for this results.

4.2 Effects of Ultrasonic Pre-Treatment on Substrate

4.2.1 COD concentration

To evaluate the effect of ultrasonic disintegration on the COD concentration in more details, total COD (after filtration through 5 µm filter paper) and soluble COD

concentrations (after filtration through 0.45 µm filter paper) were measured and compared to each other. Colloidal COD concentrations were calculated by subtracting the SCOD form total COD. The COD concentrations of untreated and sonicated samples are presented in Table 4.2 below.

Table 4.2: COD concentrations of untreated and sonicated substrates

Specific Energy (kJ/kgTS)	COD < 5µ (mg/L)	COD < .45µ (mg/L)	Colloidal COD (mg/L)
0 (Untreated Sample)	19230	7865	11365
259	19937	8950	10987
758	21652	9610	12042
1484	22615	10240	12375
2334	24225	12070	12155

Figure 4.1 shows the comparison of total, colloidal and soluble COD concentrations.

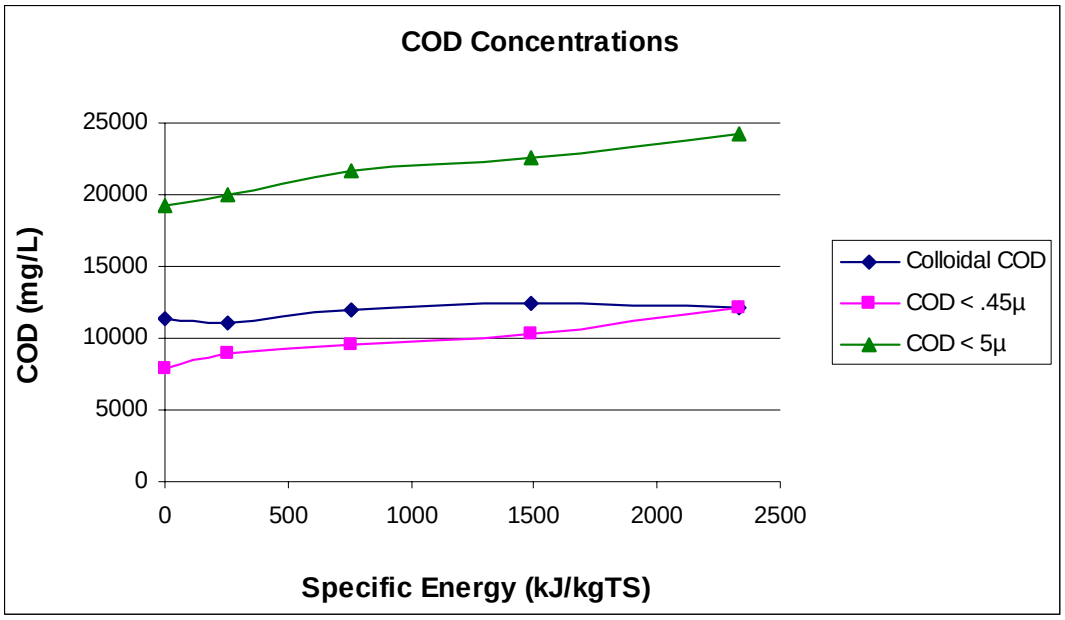

Figure 4.1: COD concentrations with respect to specific energy

COD concentration particle size less than 5µ increased with increasing sonication time, as expected. Comparing colloidal and soluble COD concentrations, the size reducing effect of ultrasound can be seen. 1 minute sonication leads to a 13.7% increase in soluble COD whereas 3.3% decrease in colloidal COD. Organic solids are destructed into smaller organic particles and these particles are further destructed

into soluble organics at the same time. Therefore the increase of soluble COD was always higher than the increase in colloidal COD.

Specific soluble COD values can also be an interesting parameter as the initial VS content of each reactor showed difference from each other. The soluble COD concentrations of each mixture prepared for biomethane potential test were measured and specific SCOD values were calculated according to VS values of the corresponded mixture. As it can be seen in Figure 4.2, the solubilization effect of ultrasonic pre-treatment was limited below 10 minutes sonication as there is no significant difference between 3 and 6 minutes sonicated samples. However, between 6 and 10 minutes sonication there is a significant increase (17%). This results comply with specific cumulative biogas productions which are presented in Figure 4.2.

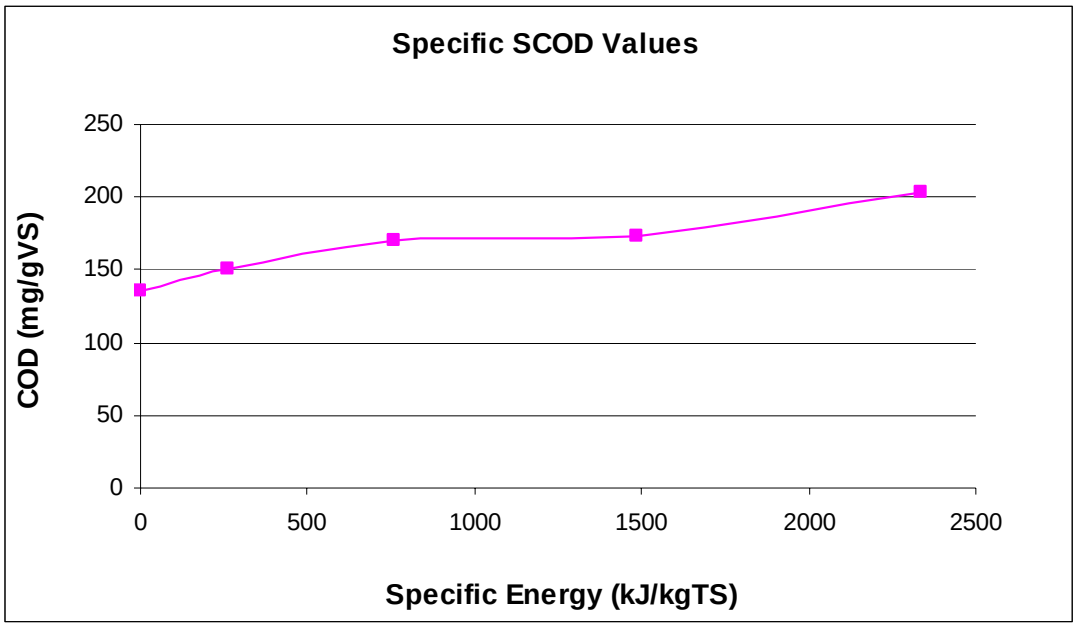

Figure 4.2: Specific SCOD values with respect to specific energy

4.2.2 Disintegration degree

According to the increase in the soluble COD concentration, the degree of disintegration was calculated. Figure 4.3 shows the relationship between degree of disintegration and sonication time. The greatest increase in disintegration degree was observed from 6 min. to 10 min. sonication. It is also observed that the increase in disintegration degree is not linear with the increase in sonication time. From 1 minutes to 3 minutes, disintegration degree increased by 60%, from 3 to 6 minutes sonication, the increase was 36% and from 6 to 10 minutes the increase was 77 %. Figure 4.1, 4.2 and 4.3. is evident that as the sonication time increases, higher

amount of soluble substances are released into the sample, so the soluble COD concentrations and disintegration degree increase.

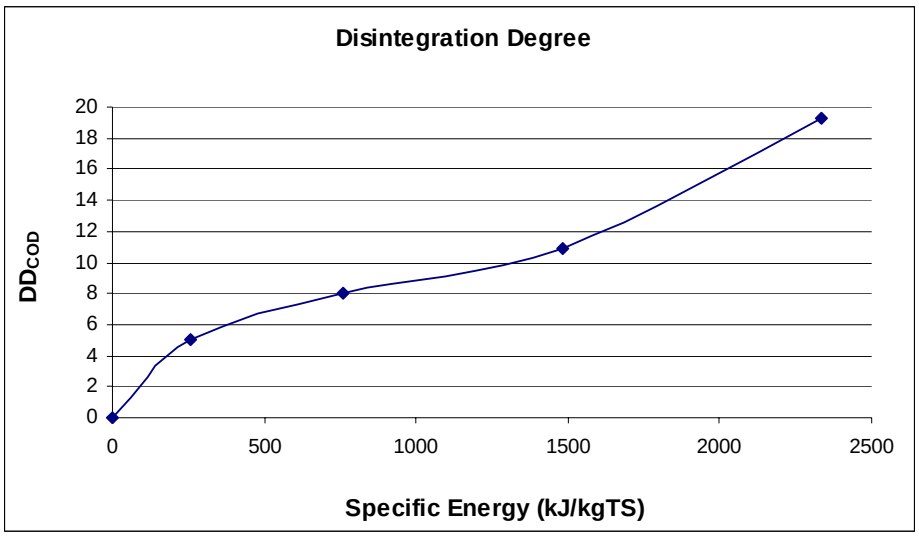

Figure 4.3: Disintegration degree with respect to specific energy

4.2.3 Specific COD to specific NH_4-N ratio

In figure 4.4, specific SCOD to specific NH4-N ratios of each inoculum and substrate mixture were presented.

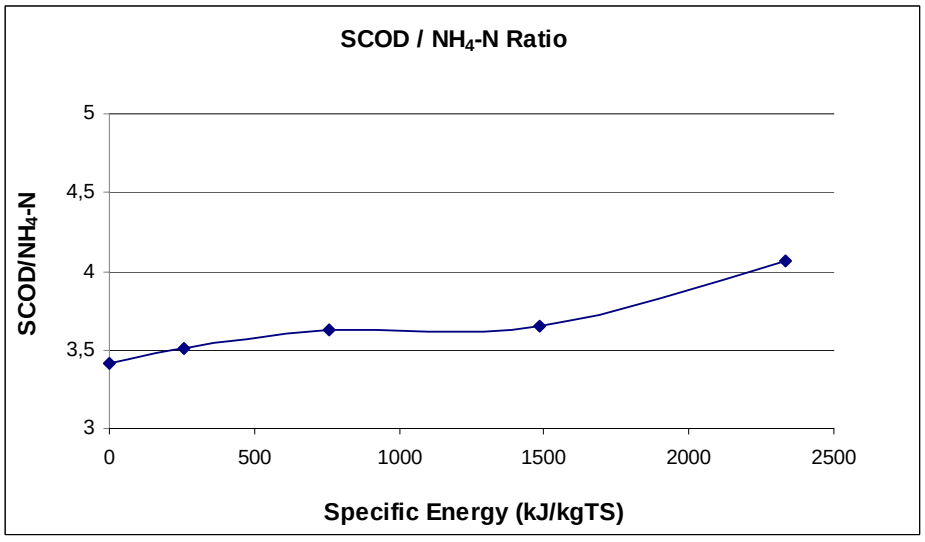

Figure 4.4: SCOD/NH_4-N ratio with respect to specific energy

4.2.4 Protein concentration

Protein concentration of untreated and sonicated samples were measured after filtration from 5 μm and 0.45 μm filter papers using Lowry's method. The results are given in Figure 4.5. Although the concentrations varied until 6 minutes sonication,

after this point a significant increase in both soluble (particle size less than 0.45 µm) and total (particle size less than 5 µm) protein concentration was observed. The increase in total protein concentration was 17% and in soluble protein concentration was 13%. This result is in compliance with the increase in soluble COD and disintegration degree and indicates that 10 minutes US treatment leads to the highest release of intracellular substances.

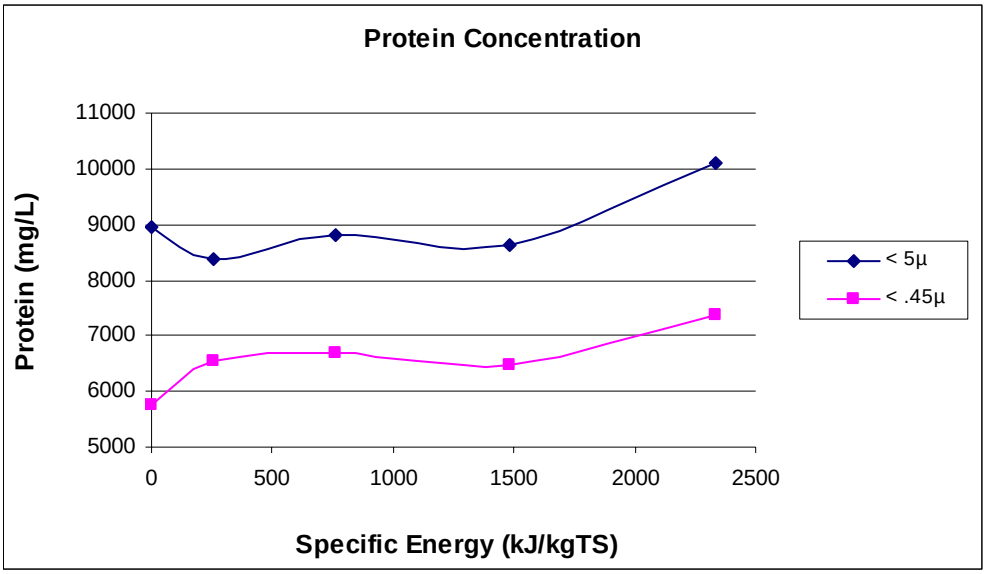

Figure 4.5: Protein concentrations with respect to specific energy

4.3 Effects of Ultrasonic Treatment on Anaerobic Digestion and Biogas Yield

To investigate the effect of Ultrasonic treatment on anaerobic digestion, biomethane potential test (BMP) was carried out. In this test, cumulative methane production was monitored for sludge and inoculum mixtures prepared with untreated and sonicated sludge samples. For comparison purpose an automatic biomethane potential test system was used. Six mixtures were prepared using cellulose, untreated sludge, 1, 3, 6 and 10 minutes sonicated sludge as substrate. A blank reactor was also employed with only inoculum to determine the gas production from inoculum itself. The test was carried out in duplicates. The substrate/inoculum ratio and total volume was kept the same in each reactor except the two with cellulose, which are 1:1 and 400 ml respectively. Because cellulose is a readily biodegradable substrate, the substrate to inoculum ratio was decided to be smaller. Produced biogas in each reactor was passed through the bottles filled with sodium hydroxide (NaOH) to fix the carbon dioxide (CO2) so that measured gas volume directly would indicate the methane volume produced. Before the start up, one extra mix of each sample was prepared to

measure TS, VS and pH. The operational parameters of each anaerobic reactor were listed in Chapter 3, Table 3.2.

For each mixture, COD, TOC, TN, NH4-N and protein concentrations were measured to be compared with the values after digestion. The characteristics of each mixture are listed in Table 4.3.

Table 4.3: Characteristics of sludge mixtures before anaerobic digestion

Mixture	COD < 5µ (mg/L)	SCOD < .45µ (mg/L)	TOC (mg/L)	TN (mg/L)	NH4-N, mg/L	Protein < 5µ (mg/L)	Protein < .45µ (mg/L)
Innoculum + Deionized Water	11220	5295	3043	1368	1490	3377	2922
Innoculum + Cellulose	-	-	2195	929	-	3624	3021
Innoculum + Untreated Substrate	20565	9225	6065	2812	2700	7842	5946
Innoculum + 1 min. Sonicated Substrate	20625	9770	6385	2683	2784	8646	5140
Innoculum + 3 min. Sonicated Substrate	20710	10100	6505	2780	2784	9924	4464
Innoculum + 6 min. Sonicated Substrate	22730	10415	7035	3272	2850	8424	6016
Innoculum + 10 min. Sonicated Substrate	23020	11330	6995	3440	2784	8658	6029

It was expected that the COD, TOC, TN and NH4-N concentrations of the samples to be increased with increasing sonication time. However, a regular increase was observed only in COD concentrations. TOC concentrations increased until 6 minutes and other parameters didn't show any significant difference. Results of the measurements show that total nitrogen concentrations were lower than ammonium nitrogen which is not possible in reality. The reason for that result could be the different measurement methods of both parameters.

The same measurement set was done after 40 days of digestion. As the maximum gas production was not reached at the end of 40 days digestion period, one of each reactor pairs kept in operation to be monitored further. And the anaerobic digestion

process was ended for the other reactors. Therefore the values measured are not the average of two reactors but direct value from one reactor. The characteristics of the sludge mixtures after 40 days of digestion are listed in Table 4.4.

Table 4.4: Characteristics of sludge mixtures after anaerobic digestion

Mixture	COD < 5µ (mg/L)	SCOD < .45µ (mg/L)	TOC (mg/L)	TN (mg/L)	NH4-N, mg/L	Protein < 5µ (mg/L)	Protein < .45µ (mg/L)
Innoculum + Deionized Water	7535	4060	2920	1290	1404	4333	3809
Innoculum + Cellulose	9580	5275	3060	1505	1905	4822	3255
Innoculum + Untreated Substrate	22065	8380	3115	2745	3660	9078	6851
Innoculum + 1 min. Sonicated Substrate	18905	8060	3150	2450	3390	7800	6007
Innoculum + 3 min. Sonicated Substrate	21050	8010	3155	2770	4050	9014	6812
Innoculum + 6 min. Sonicated Substrate	20680	7730	3165	2790	3540	8846	6698
Innoculum + 10 min. Sonicated Substrate	19320	8375	3065	2840	3480	8345	6567

A significant reduction in soluble COD and TOC concentrations was observed. Protein concentrations particle size less than 5µm did not changed significantly. For the protein analysis the samples had to be diluted with a dilution factor of 50. This high dilution factor can have an impact on the accuracy of the results. Eventhough, a significant increase in soluble protein concentrations after anaerobic digestion was observed.

Specific Ammonia nitrogen (NH4-N) concentrations were calculated for each mixture and the values before and after digestion were compared in Figure 4.6. There was 21 to 35 % increase in NH4-N concentrations after digestion which proves the cell lysis.

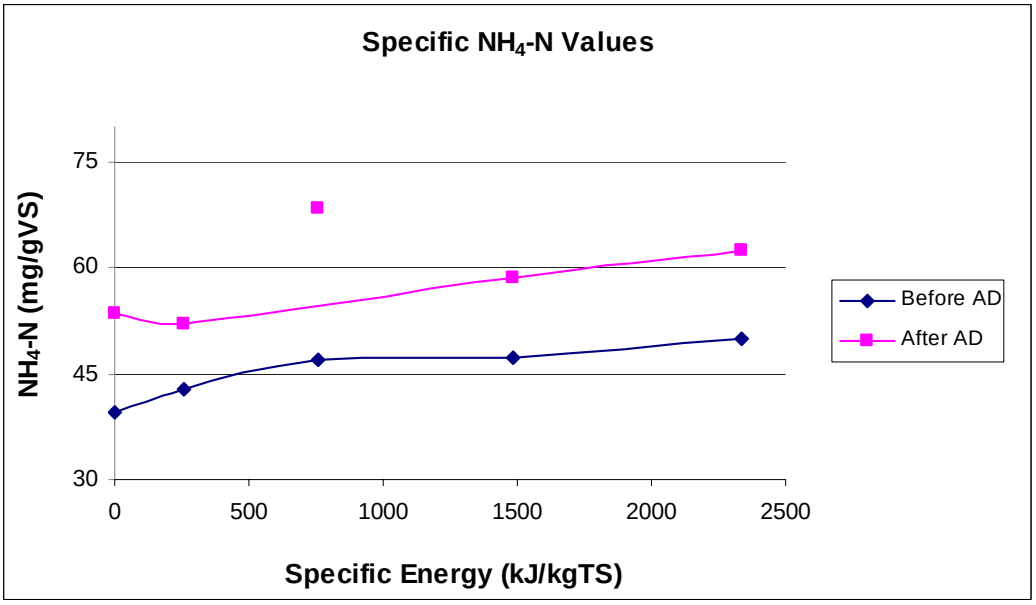

Figure 4.6: Specific NH_4-N concentrations with respect to Specific energy before and after digestion

In Figure 4.6. gVS represents the total VS mass in each anaerobic reactor measured before digestion. The final NH4-N concentration of the 3 minutes sonicated sample was unexpectedly high, therefore, it was considered as an error.

After anaerobic digestion, soluble COD concentrations of each mixture were measured and compared with the ones measured at the beginning of the experiment. Figure 4.7 shows the SCOD concentrations before and after anaerobic digestion process with respect to specific energy. As it can be seen in the figure, the final concentrations of SCOD were closed to each other and at the level around 8000 mg/L which is the SCOD level of untreated substrate at the beginning. Ultrasonic treatment increased the solubilization hence, biodegradability of the sludge. Increased concentrations of SCOD was observed to be reduced and no further reduction was observed within 40 days of digestion.

Figure 4.8 shows the SCOD reduction in each anaerobic reactor. The SCOD reduction increased with sonication time. It was expected that more reduction would be observed in 10 minutes sonicated sludge sample whereas the increase in reduction from 6 minutes sonication to 10 minutes was not very high.

Biogas volumes produced during the anaerobic digestion process was monitored continuously using an automatic biomethane potential test system. Because the test was performed in duplicates, the average values of two reactors were calculated for

each mixture. Figure 4.9 shows the cumulative biogas production of only inoculum and inoculum + cellulose mixture during 40 days of digestion.

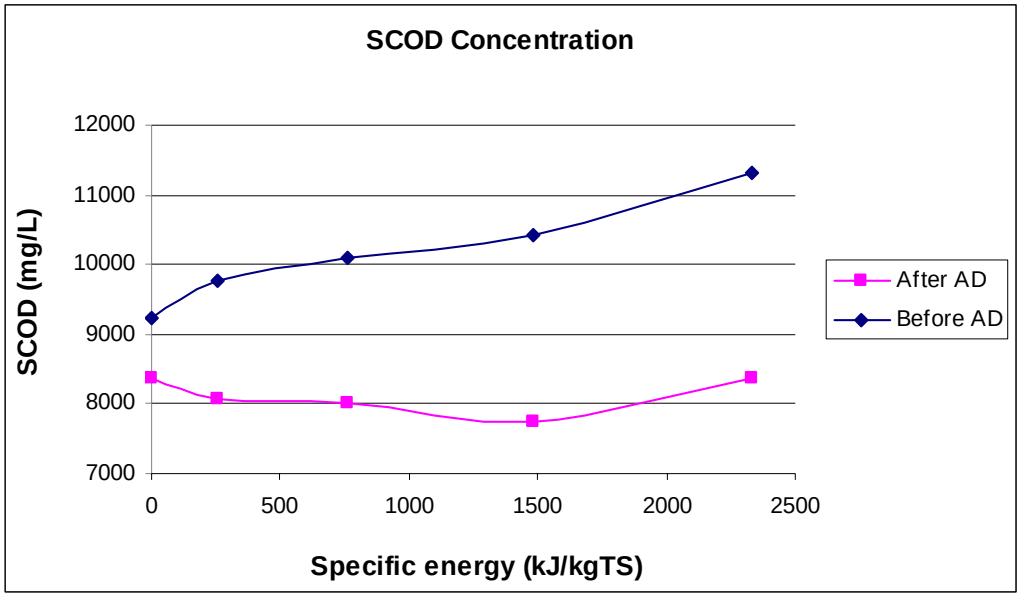

Figure 4.7: SCOD concentrations with respect to Specific energy before and after digestion

According to the VDI standards, with 100 % conversion and taking the new formation of biomass into consideration, a gas quantity would be produced from microcrystalline cellulose of 740 to 750 Nml/gVS. This value should be 80 % reached in the control batch to assume that the biologically active mass has an adequate level of potential performance (VDI 4630).

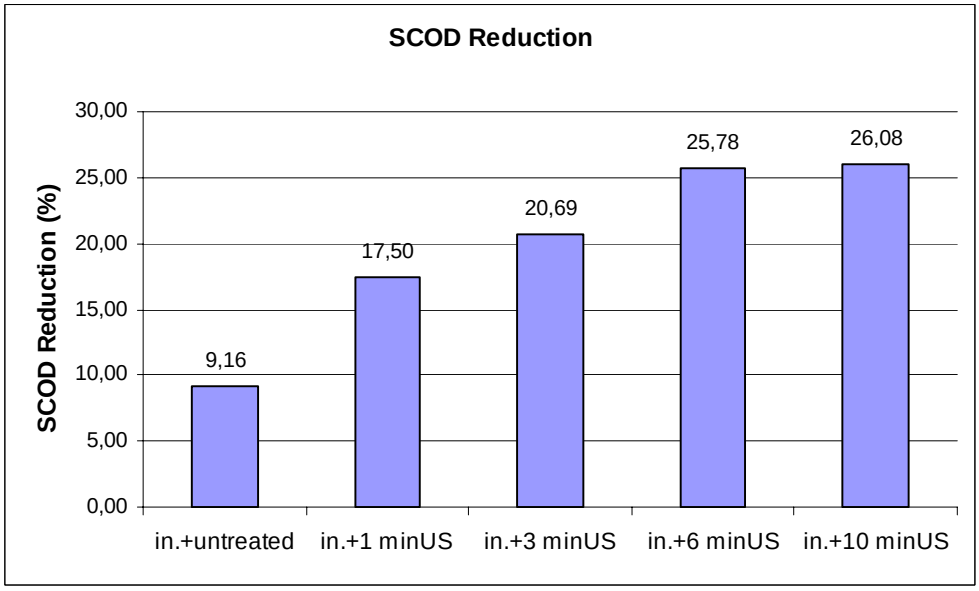

Figure 4.8: Volatile solids (VS) reduction after 40 days of digestion

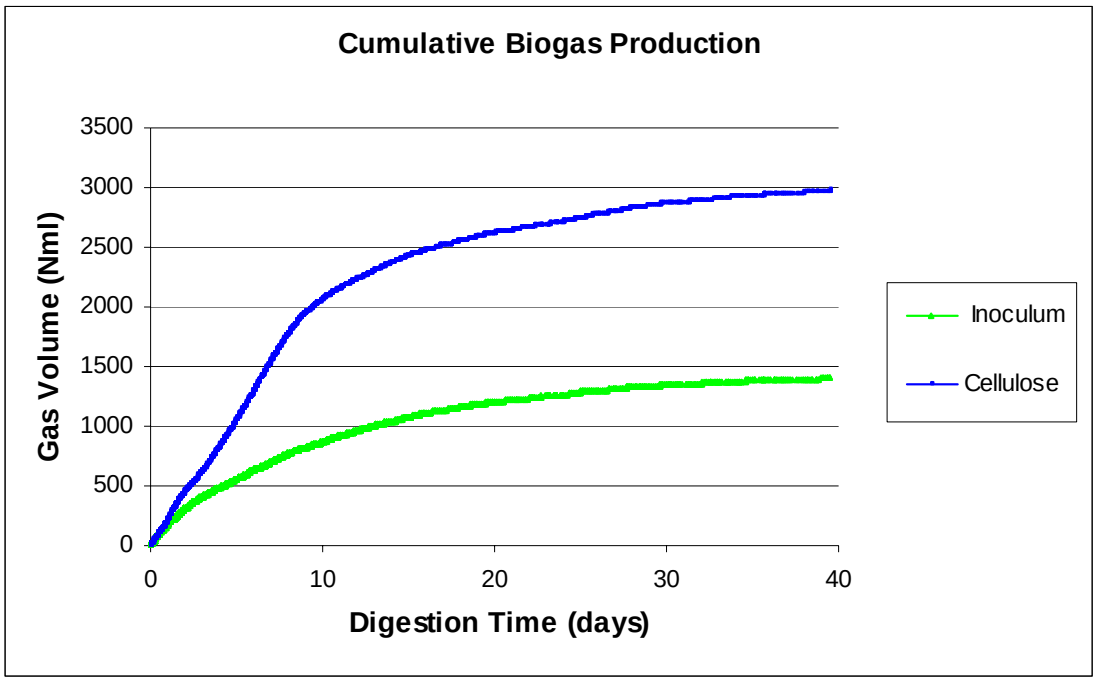

Figure 4.9: Cumulative biogas production from inoculum and inoculum + microcrystalline cellulose

Substracting the gas produced from inoculum itself, it can be calculated that 425 Nml of gas produced per gram cellulose added. This value is less than the accepted value in VDI standars, however, it should be notted that the CO_2 fixation was employed in BMP tests so the gas values measured are less than total biogas volumes. Therefore it can be asssumed the biological activity of the inoculum is sufficient for BMP test.

The average specific biogas volumes of each reactors were shown in figure 4.10. As the same amount of inoculum was used in all of the reactors, total biogas volumes are directly comparable with each other. The specific biogas volumes were calculated according to total VS contents of inoculum and substrate in the reactors.

As it can be seen in figure 4.10, the specific biogas volumes are very small which indicates that the biodegradability of the sludge was very low. The reason for that is the substrate is already digested residual biomass which contains high amounts of hemi-cellulose and lignin. However, there was a significant improvement after ultrasonication. The maximum biogas production observed in 10 minutes. sonicated sluge which was 21% higher than untreated sludge. The increase in gas production of 1 minute sonicated sludge was 4.5% whereas the increase in 3 and 6 minutes sonication was 13 and 11% respectively.

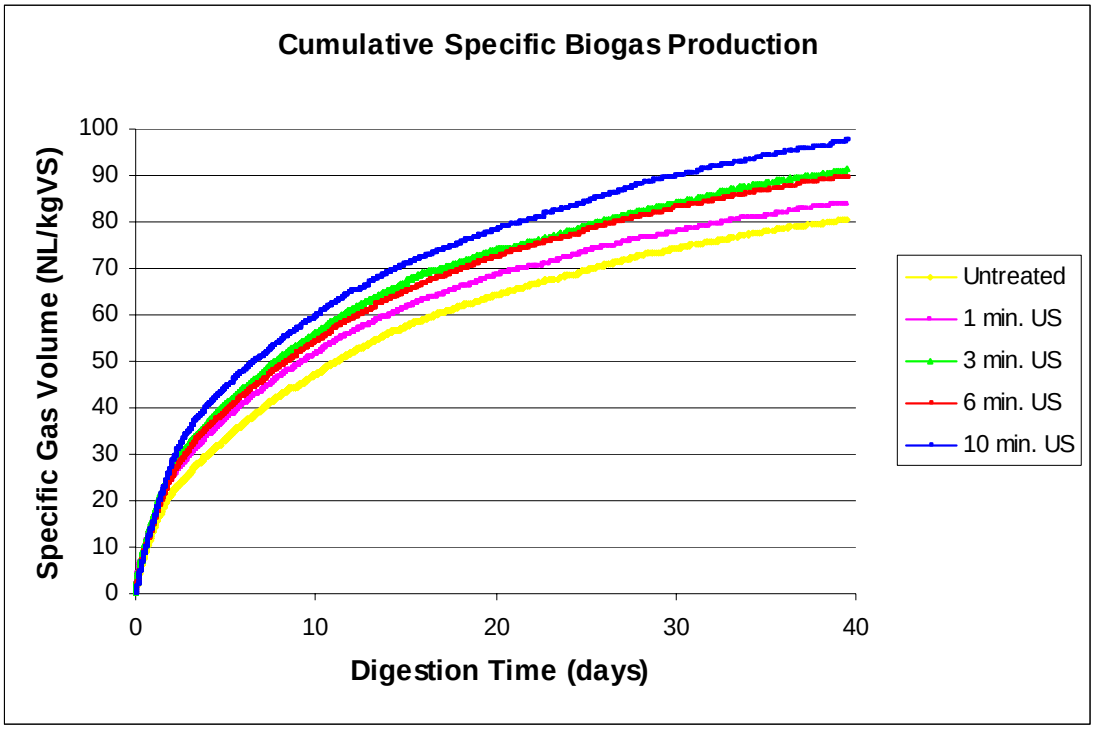

Figure 4.10: Cumulative specific biogas production curve of untreated and 1, 3, 6, 10 minutes sonicated sludge samples from the farmland biogas plant

As it can be seen in figure 4.10, the specific biogas production curves ofuntreated and sonicated sludge from the farmland biogas plant did not reached the maximum level at the end of 40 days. In the digestion of waste activated sludges of wastewater treatment plants, most of the biogas production is completed during the first week of digestion process.

Another Biomethane Potential (BMP) Test was applied to the digested sludge from Seevetal Wastewater Treatment Plant, Germany, using the same BMP test system and can be an example for comparison with the results of the digested sludge samples from farmland biogas plant. For this test, mixtures with untreated, and 1 and 5 minutes sonicated sludge samples with 1/1 inoculum to substrate ratio was prepared and the test was carried out for 28 days. Because this test was carried out only to test the automatic BMP test system itself, the complementary analysis were not done and can only be an example to compare gas production curves. Figure 4.11. shows the cumulative specific biogas production of this test. 5 minutes sonication increased the gas production by 38%. In this test, approximately 90% of biogas production was completed within 10 days for 5 minutes sonicated sludge and 13 days for untreated and 1 minute sonicated sludge.

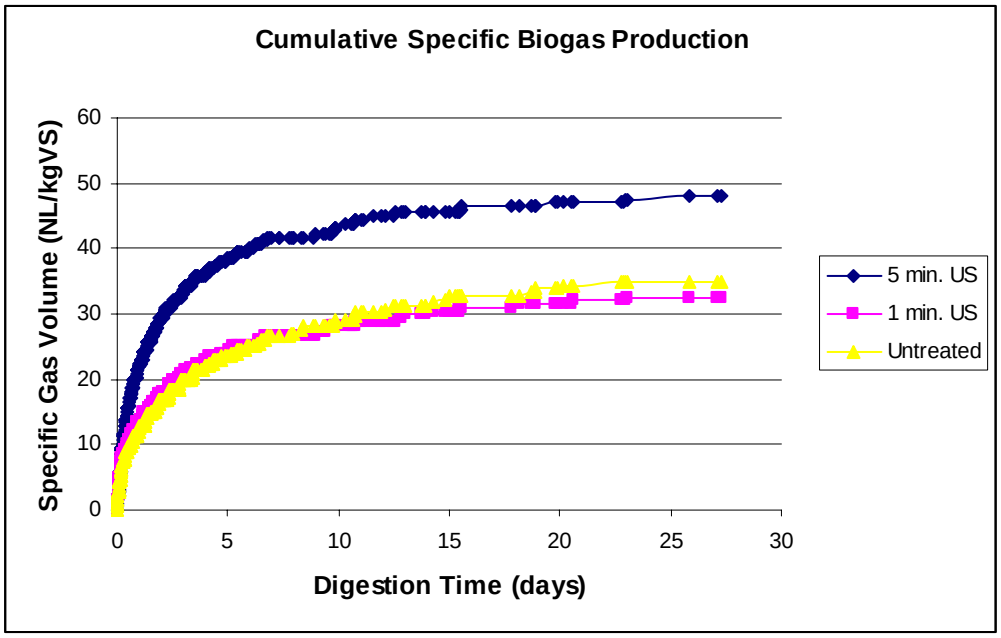

Figure 4.11: Cumulative specific biogas production curve of untreated and 1, 5 minutes sonicated sludge samples from a WWTP

Comparing the figures 4.10 and 4.11 it can be seen that further digestion of digested sludge of the farmland biogas plant is possible, however, the process is very slow and longer retention times in digesters are needed.

5. CONCLUSIONS

In this study the effect of ultrasonic pre-treatment on residual biomass of a farmland biogas plant was examined. From the experimental results, it can be concluded that ultrasonic pre-treatment shows a positive effect on digestibility of the lignocellulosic biomass.

Soluble COD before and after sonication showed that the solubilization of organic mattet increases with increasing specific energy input. Ten minutes sonication caused 4200 mg/L increase in soluble COD compared to untreated sample. The soluble COD increase was directly proportional to the specific energy.

Biomethane potential tests showed that further digestion of digested sludge of farmland biogas plant is possible, however it takes longer digestion time compared to waste activated sludge or digested sludge of a wastewater treatment plant as after 40 days of anaerobic digestion, the biogas volumes continued to increase.

Ultrasonic pretreatment has not shown significant effect on digestion time of the farmland biogas plant sludge, however a significant increase in biogas production has been observed. 10 minutes digestion (2334 kJ/kgTS) caused 21% increase in biogas volume produced.

Ultrasonic pretreatment of digested sludge of a WWTP has both decreased digestion time and increased biogas production significantly. There was a 30% increase in biogas production after 5 minutes sonication. 90% of biogas production was complated earlier with the sonicated sample than untreated one.

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